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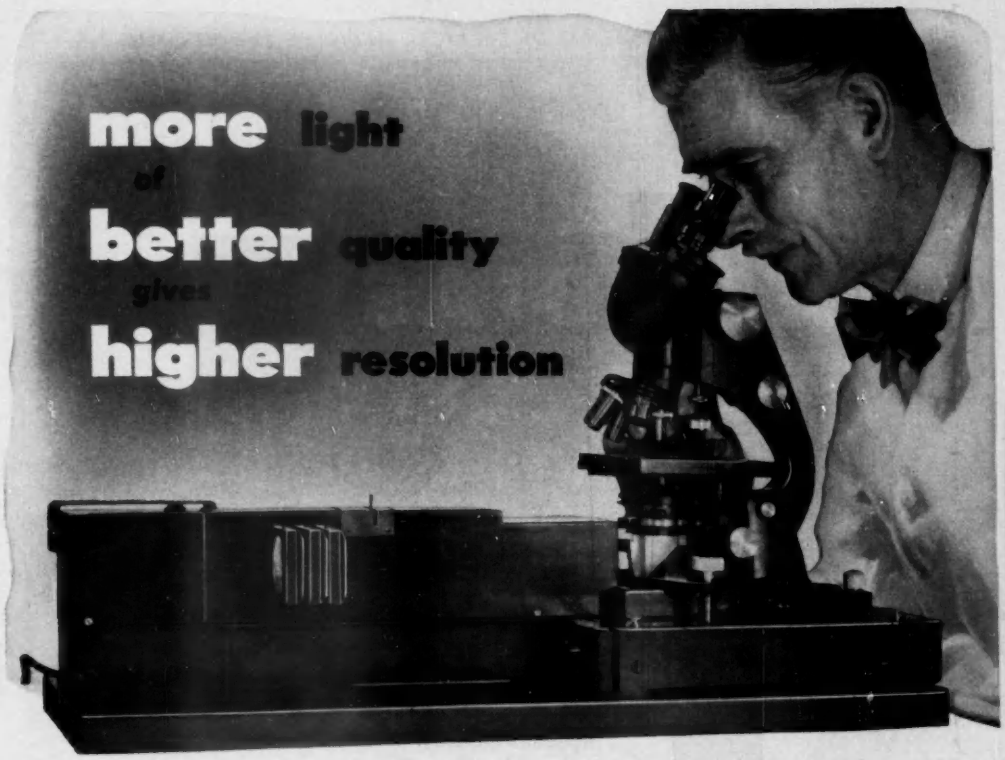

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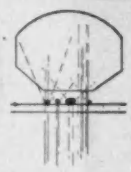

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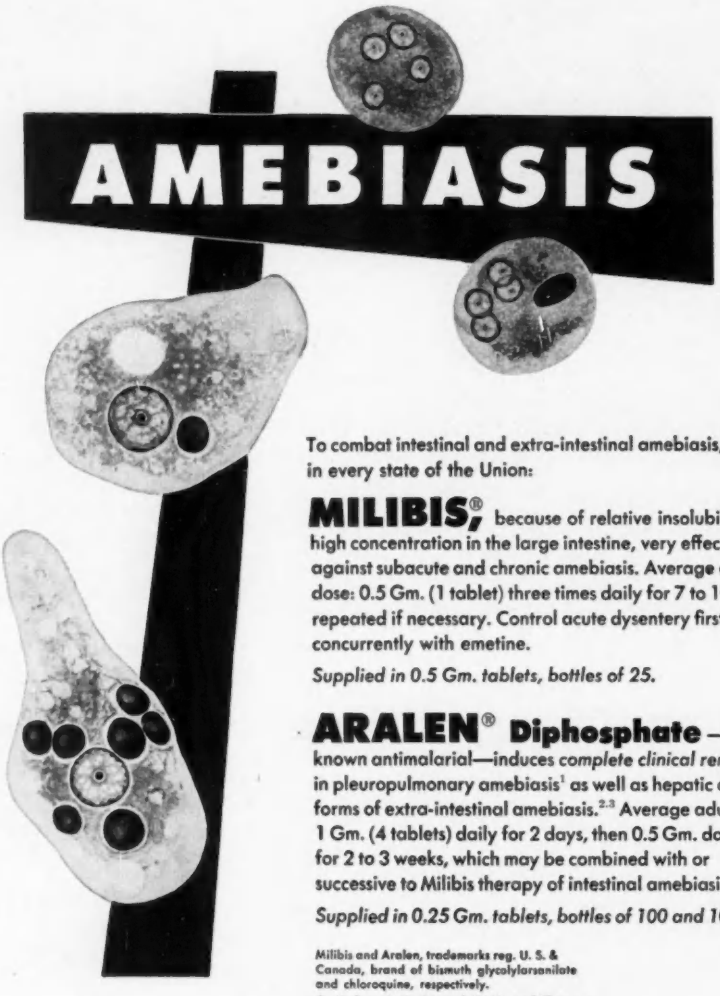

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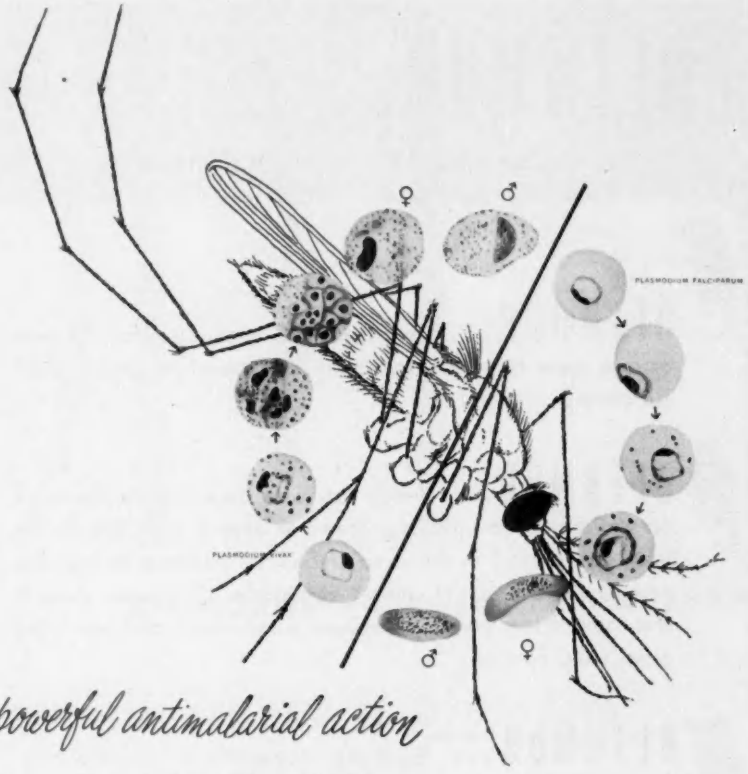

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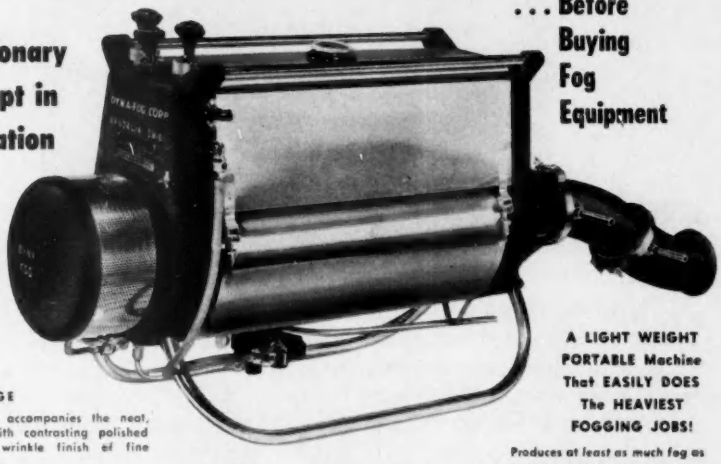

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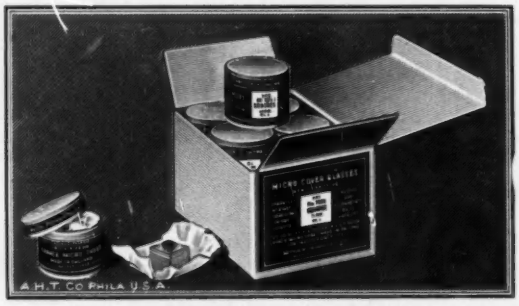

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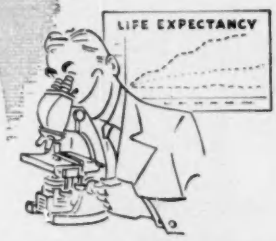

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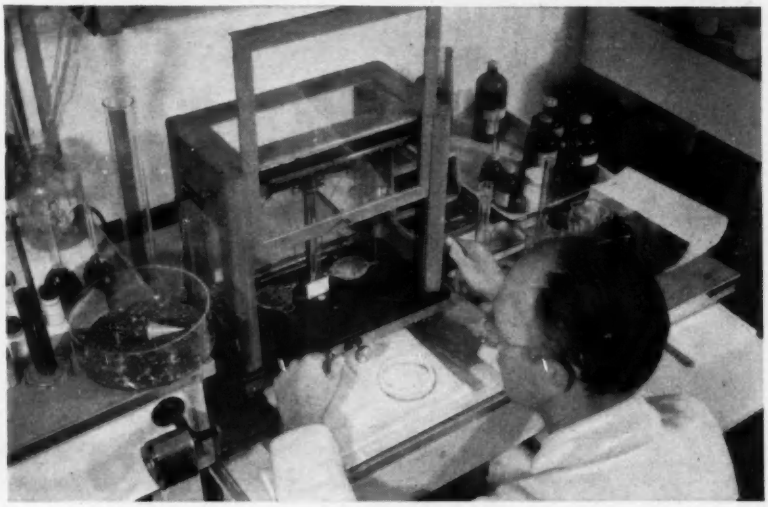

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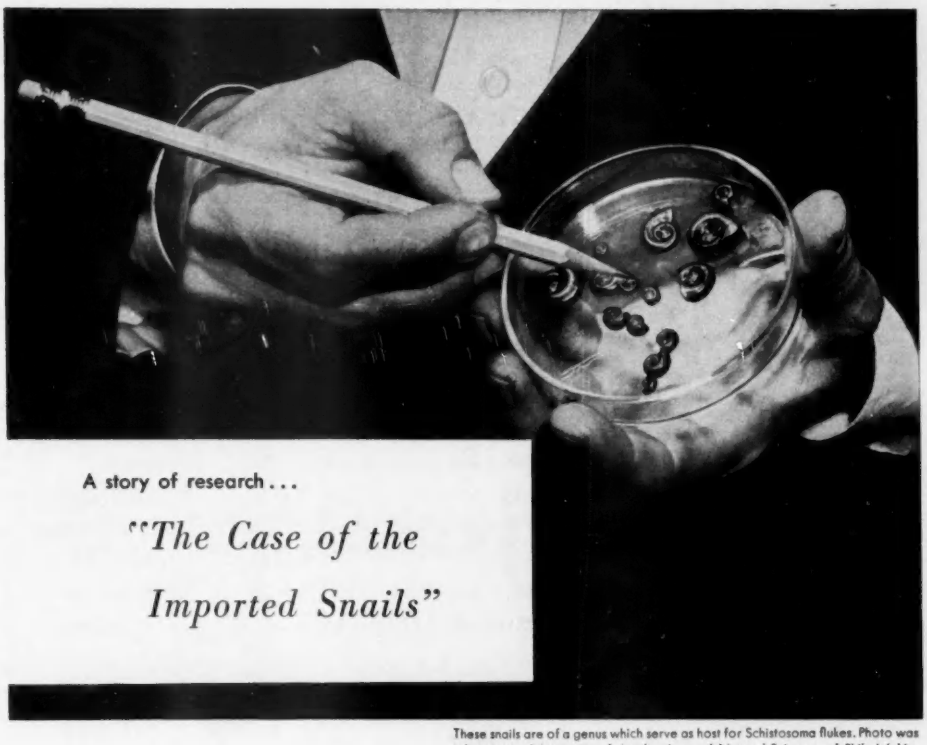

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EXO-ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM*

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INTRODUCTION

The experiments reported in the present paper were designed to determine if exo-erythrocytic stages of *Plasmodium falciparum* could be found in the liver. Tissue phases in the liver had been described previously for *P. vivax* and *P. cynomolgi* (Shortt and Garnham, 1948; Hawking, Perry, and Thurston, 1948).

In December, 1948, the present work was undertaken. Before the completion of the experiments involving 14 patients, Shortt and his collaborators (1949, 1951) reported an experiment with a single patient wherein they found in the liver bodies designated as pre-erythrocytic parasites of *P. falciparum*.

MATERIALS AND METHODS

1. *Massive inoculation of patients*

The parasite used was a strain of *P. falciparum* isolated from a mestiza in Panama in 1948 and designated in this laboratory as Pf-6. Three strains of *Anopheles* were used in the massive inoculations: *A. quadrimaculatus* (Q-1) and the Panama (A-2) and Florida Keys (A-3) strains of *A. albimanus* (Rozeboom, 1936; Burgess, 1950). *A. albimanus* was used principally, as this species has a higher susceptibility than does *A. quadrimaculatus* to the strain of *P. falciparum* used (Jeffery, Eyles, and Young, 1950). For a similar reason, there were about twice as many Panama *A. albimanus* used as the Florida Keys strain.

Large numbers of mosquitoes were fed on good *P. falciparum* gametocyte carriers and maintained singly in celluloid cylinders until sporozoites were in the glands. Samples of these mosquitoes were dissected during the oocyst stage to determine the degree of infection. In a few of the lots used only a very few dissections were done, the suitability of the lot for use being determined by the detection of heavily infected specimens in the first two or three specimens examined. The mosquito infection data are summarized in table 1.

Ordinarily, only the most heavily infected lots of mosquitoes were used. In a few cases, some mosquitoes from the less heavily infected lots were used. This means that the infection rates of the mosquitoes actually used are higher than indicated in table 1.

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We express our appreciation to Dr. Edward Cardwell, pathologist, Veterans Administration Hospital, Columbia, South Carolina, and Dr. James H. Peers, Pathology Laboratory, National Institutes of Health, Bethesda, Maryland, for their critical examination of some of the preparations.

Patients selected for malaria therapy and biopsy were given sporozoite inoculations by two methods. Of a total of 14 patients, eight were inoculated through the bites of infected mosquitoes only, three by intravenous inoculation of infected salivary glands, and three by a combination of both methods. Inoculations were done over a period of from two to six days. Table 1 summarizes

TABLE 1

Summary of inoculation and biopsy data in 14 cases included in exo-erythrocytic studies on P. falciparum

PATIENT NUMBER	INOCULATION				INOCULATION DAYS (b)	BIOPSY		FIRST PARASITE DAY (c)
	Per cent of mosquitoes infected	Number of bites	Glands (a)			Day after inoculation	Method	
			Number	Inoculation medium				
1	74.2	544	0	—	5	3 to 7	Peritoneoscope	11
2	74.1	473	0	—	4	2 to 5	Peritoneoscope	13
3	74.1	733	0	—	5	2 to 6	Peritoneoscope	11
4	74.1	379	0	—	3	2 to 4	Peritoneoscope	12
5	68.2	386	0	—	2	2 to 3	Peritoneoscope	9
6	82.0	587	0	—	4	4 to 7	Peritoneoscope	9
7	82.4	518	0	—	3	5 to 7	Peritoneoscope	9
8	82.0	1,246	0	—	6	2 to 7	Peritoneoscope	9
9	80.7	297	44	Whole blood (d)	2	3 and 6	Peritoneoscope	9
10	67.6	0	116	Whole blood	2	6 to 7	Peritoneoscope	7–8 (e)
11	92.6	0	227	Whole blood	2	7 to 8	Laparotomy	7
12	91.4	171	181	Whole blood	4	4 to 7	Peritoneoscope	7
			86	1N saline				
13	80.0	0	651	Locke's (f)	4	3 and 5 to 7	Peritoneoscope	7
14	86.8	8,516	1,403	Defibrinated blood	4	3 to 6	Laparotomy	7

(a) Glands were dissected from mosquitoes in sterile normal saline and injected intravenously.

(b) Number of days on which inoculations were done.

(c) Days after first inoculation.

(d) Whole blood was citrated in all cases.

(e) No blood smear made on day 7. Probably positive at that time.

(f) Modified Locke's solution consisted of ½ Locke's and ½ normal saline.

the inoculation data on the 14 patients included in the study, and table 2 illustrates the inoculation and biopsy schedules in the 14 cases.

The inoculation techniques were as follows:

A. *Inoculation by bites of mosquitoes.*

The infective specimens were fed on the recipients using the interrupted bite method to ensure a maximum of infective bites from the available mosquitoes.

In using multiple bites the mosquito was allowed to penetrate and actually begin feeding before removing for another bite. By previous experiments in this laboratory it has been shown that penetration of the skin by a mosquito, for

only a second or two but not long enough for the mosquito to withdraw enough blood for it to be visible in the abdomen, is sufficient for the mosquito to inject enough sporozoites to cause a typical infection of malaria. A total of 5,034 bites was administered to the first nine patients inoculated (table 2) using a total of 589 mosquitoes, with an average of 8.6 bites per mosquito. In accomplishing the 8,516 bites on the last patient (No. 14, table 2) there was an average of 15.4 bites per mosquito. In general the average number of bites per mosquito increased with the total number of bites on the patient. For example, patient 9 with 297 bites received an average of 3.1 bites per mosquito as compared with the 15.4 average for patient 14.

B. *Intravenous inoculation of salivary glands.*

Infected mosquitoes used as a source of salivary glands were taken from the same groups as those which were used in administering bites on the patients. Frequently mosquitoes which had already bitten patients were used also as a source of infective salivary glands.

The mosquitoes were anaesthetized lightly with chloroform and transferred to sterile microscope slides under the dissecting microscope. Using sterile needles the head was severed and the glands expressed in a drop of sterile normal saline by pressure on the thorax. The glands were separated from the usual debris and transferred with a needle to a large drop of sterile normal saline in a hollow-ground slide. When a number of glands (usually 10 to 15) had been transferred to this saline they were taken into a one-half cc. hypodermic syringe and transferred to a vaccine bottle containing 5 to 10 cc. of the medium for inoculation. The aspiration of the glands from the hollow-ground slide was done under the dissecting microscope and was followed by frequent washings of the slide and syringe with more sterile saline. When enough glands had been collected in the inoculation medium it was drawn into a 10 cc. syringe and injected intravenously. The vaccine bottle and syringe were washed two to four times with sterile saline and the washings also injected intravenously. The glands were kept in the suspension for only short periods of time, never exceeding two hours.

Whole citrated blood was used as a carrier for injection of the glands in four cases (table 1). In one of these cases (No. 12) some of the glands were suspended in normal saline only. In one case (No. 13) all glands were suspended in a moidfied Locke's solution, prepared with equal parts of Locke's solution and normal saline. In the last case (No. 14) the glands were suspended in defibrinated blood for injection.

Penicillin was given all recipients of gland injections to prevent the establishment of bacterial infection through the inoculation technique. The penicillin was given either intravenously (100,000 units penicillin G-crystalline-potassium) following each gland injection, or a blood level was maintained by use of procaine penicillin-G in oil intramuscularly during the course of inoculation.

2. *Surgical procedures for obtaining liver biopsies*

Liver tissue was removed from each of the 14 patients, from 12 by the use of a peritoneoscope and from two by laparotomy. The standard peritoneoscope of

TABLE 2

Schedules of inoculations with P. falciparum *sporozoites by mosquito bites and glands and of biopsies*

PATIENT NUMBER	DAYS OF INOCULATION														BIOPSY Day (b)
	Day 0 (a)		Day 1		Day 2		Day 3		Day 4		Day 5		Total		
	Bites	Glands	Bites	Glands	Bites	Glands	Bites	Glands	Bites	Glands	Bites	Glands	Bites	Glands	
1	100	0	89	0	69	0	161	0	125	0	0	0	544	0	7
2	117	0	117	0	115	0	124	0	0	0	0	0	473	0	5
3	115	0	86	0	133	0	240	0	159	0	0	0	733	0	6
4	162	0	99	0	118	0	0	0	0	0	0	0	379	0	4
5	181	0	205	0	0	0	0	0	0	0	0	0	386	0	3
6	74	0	200	0	140	0	173	0	0	0	0	0	587	0	7
7	123	0	206	0	189	0	0	0	0	0	0	0	518	0	7
8	74	0	207	0	156	0	202	0	300	0	307	0	1,246	0	7
9	297	0	0	0	0	0	0	44	0	0	0	0	297	44	6
10	0	56	0	60	0	0	0	0	0	0	0	0	0	116	7
11	0	102	0	125	0	0	0	0	0	0	0	0	0	227	8
12	79	99	0	82	92	46	0	40	0	0	0	0	171	267	7
13	0	242	0	150	0	121	0	0	0	138	0	0	0	651	7
14	869	233	2,206	347	3,297	585	2,144	238	0	0	0	0	8,516	1,403	6

(a) Day 0 in all cases is the first day of inoculation.

(b) Day of biopsy corresponds to the number of elapsed days from the first day of inoculation.

the American Cystoscope Makers, Inc.[2] was used. Sodium pentothal inductions and local infiltrations were used as anesthesia.

Peritoneoscopy was performed through a routine stab-blade incision at a point one and one-half inches above the umbilicus and one inch lateral to the right in each of the 12 patients. Using biopsy forceps through the peritoneoscope sheath, tissue was taken from the inferior border of the liver, usually within one centimeter of the mid-clavicular line. In most cases two biopsies were taken with the forceps. Electric cautery and routine wound closure followed. The size of the specimens obtained by this method varied from about the size of a grain of wheat to that of a small pea.

Laparotomy was performed on two patients to obtain tissue samples. A right mid-rectic incision was made, extending from the costal arch to approximately one or two centimeters above the umbilicus. The liver was exposed and a pyramidal biopsy taken approximately one inch across at the base and three-fourths inch long in the case of patient 14. The sample taken from the other laparotomized patient was about one-half this size. The liver was closed routinely with mattress sutures; bleeding was stopped; the routine type of wound closure was used.

3. *Micro-technique*

The liver material was fixed immediately in Carnoy's fluid, using the following proportions: three parts chloroform, two parts absolute ethyl alcohol, and one part propionic acid. The fixing fluid was changed several times during the first hour and the total time of fixation was seven hours. The specimens obtained by laparotomy were cut into smaller pieces to obtain better penetration of the fixative. Following fixation the material was dehydrated using n-butyl alcohol to lessen shrinkage and was passed gradually from pure n-butyl alcohol to paraffin and embedded. Routine serial sections were cut at six microns, but some sections were cut at two and three microns.

Most of the sections were stained with a colophonium-Giemsa method (Shortt and Cooper, 1948). Some sections were stained by other methods including Heidenhain's and Delafield's hematoxylin and Feulgen's reagent.

All tissue sections were examined at least once under low power objective (100×). All negative material was subjected to at least two further examinations using higher magnifications. All objects suspected of being of parasitic origin were routinely examined under oil immersion objective (950×).

RESULTS

Intensive examination of the material derived from the first 13 cases resulted in entirely negative findings. Four-fifths of the material from patient 14 has been examined and 125 parasites have been found. About 100 of these parasites are complete, i.e. with no sections missing, and these received the most attention.

[2] The peritoneoscopes were very kindly provided by the following: Medical Officer in Charge, U. S. Marine Hospital, Baltimore, Maryland; Dr. Lester D. Odell, Department of Obstetrics and Gynecology, The Chicago Lying-in Hospital, Chicago, Illinois.

The descriptions which follow are based on parasites stained by the colophonium Giemsa schedule, although the morphology of the parasite appears the same regardless of the stain used.

Description of Parasites

The smallest parasites seen were 15 microns in longest diameter. However, in all those seen shrinkage was present and the living parasite was undoubtedly larger than 15 microns. The smallest number of nuclei was about 40. Figures 1 and 7 are of a small parasite, figure 1 being of one section and figure 7 being of the only other section. The cytoplasm is granular, though not coarsely so. Small vacuoles may sometimes be present. The outline of the parasite appears wavy, probably due to shrinkage. A part of the host cell nucleus may be seen in figure 7.

Figures 2 and 3 show somewhat larger parasites, about 25 microns in longest diameter. Parasites in this size range differ very little from the smaller ones except in size and in larger numbers of nuclei. The cytoplasm is homogeneous and contains a few vacuoles.

Figure 4 illustrates a larger parasite (about 40 microns) which may be nearing the mature stage. Parasites of this general size seldom show shrinkage. The cytoplasm is vacuolated. The outline of the parasite is smooth and there may be the formation of small lobes. These are probably the result of uneven growth of the parasite.

A stage which is probably very near maturity is shown in figure 5. There is an increase in complexity of the vacuoles and an apparent formation of cords and islands of cytoplasm (the "pseudocytomeres" of Shortt, *et al.*, 1951); it is from these that the merozoites form. Figure 6 illustrates a mature schizont with some merozoites differentiated and ready for release. Most parasites at this stage are 55 to 60 microns in longest diameter. One long, narrow parasite was about 70 microns in length.

Location of Parasites and Effect on Host Tissue

The parasites are present in the parenchyma tissue of the liver, lying within the liver cords. The limit of the parenchyma cells is difficult to follow but in all cases where shrinkage was not pronounced the parasite was in contact with parenchyma tissue. Figures 4 and 11 show clearly that the parasite is within the liver cord.

The host cell becomes enlarged as may be seen in figure 10 (this parasite is considerably shrunken, leaving the space it had occupied and showing thus the extent of the hepatic cell).

No compression of hepatic tissues surrounding the parasites was found. There was no change in the host cell nuclei, the parasite simply growing around them (figures 9 and 10). The host cell nuclei apparently do not increase in number.

The distribution of the parasites in the liver tissue is apparently at random. Parasites have been found directly against the wall of a blood vessel (figure 9) but are not usually so located.

There is, then, no morphologically detectable reaction on the part of the host

tissue to the parasite other than a simple distention of host cells containing parasites, without any accompanying compression of the host cell nuclei or cytoplasm. There was no white cell infiltration in the areas around the parasites.

Time of appearance of erythrocytic parasites

Erythrocytic parasites were not found in the routine examination of 0.1 cmm. of peripheral blood on the day of liver biopsy from patient 14. They were first found on the following day, seven days after the first inoculation, with a density of 50 per cmm. The parasite prepatent periods for all of the patients are shown in table 1.

DISCUSSION

All of the evidence that we have found indicates that the bodies described above represent exo-erythrocytic phases of *P. falciparum*. The parasites were found in the liver after an extremely heavy infecting dose of sporozoites. Various sizes of parasites were found indicative of a natural growth pattern. The discrete particles which we interpret to be nuclei in the exo-erythrocytic bodies gave a positive Feulgen reaction, a generally accepted specific test for chromatin. The number of nuclei increased with the size of the parasites until maturation occurred with formation of merozoites, a process compatible with the schizogonic characteristics of malarial parasites. The bodies are inside the parenchyma cells, indicating parasitism.

The possibility of the bodies being other than malaria was considered. Morphologically, the parasites are different from *Toxoplasma* and other parasitic protozoa. Slides containing the bodies were examined by two pathologists who had had much experience, one in a large hospital laboratory and the other in a research laboratory. Their opinions were that these bodies were not white thrombi, were not of bacterial origin, and were not artifacts. They indicated that they found nothing to negate the possibility of these bodies being of parasitic origin. They also believed the bodies to be located within the hepatic cells and not in either the sinusoids or the reticulo-endothelial cells.

The parasites described are undoubtedly similar to those described by Shortt, *et al*, (1951). Few differences were noted, and these may be due to interpretation.

In our sections there was very little, if any, compression of host tissues in the areas surrounding the parasites. The accompanying photographs demonstrate this lack of compression. Although Shortt, *et al*, (1951) indicated that tissue compression was usually associated with the parasites, their illustrations do not show any extensive compression.

The tendency toward lobosity in the parasites, as described by Shortt was noted in our parasites. It was not unusual, however, to find parasites, even mature schizonts, with quite smooth outlines, although in many other cases the lobose character was extremely pronounced (figure 8). It appears that the parasite grows along the liver cords, branching with the cords when such branches occur, (figure 12) until it reaches its maximum size. Because of the absence of compression of cell or nucleus one might suggest that the parasite grows into adjacent

cells along the liver cord rather than between them. Cell outlines in the parenchymal liver tissues are indistinct, at best, thus making interpretation of the parasite growth mechanism difficult.

Because the inoculation of patient 14 extended over a period of four days, three to six days previous to tissue sampling, it is not possible to designate a particular parasite as a result of inoculation at any given time, i.e., we cannot specifically ascribe an age to any parasite. Shortt, *et al* (1951), also inoculated their patient on three consecutive days and have correlated their exoerythrocytic parasites with the elapsed time, designating them as being four, five, and six days of age on the basis of size; this assumes that all stages found represented consecutive stages of growth in a single cycle. The only evidence that we have in support of this hypothesis is that the smallest parasites we found were 15 microns in diameter and could have been three days old, whereas the smallest parasites found by Shortt, *et al*, were about twice that large and were designated by them as being four days old.

The parasites that we found appeared to be representative stages indicative of a continuous growth. Starting with the smallest parasites, there is a gradual increase in size and in the number of nuclei. Vacuolization of the cytoplasm increases and leads to the formation of cords or islands of cytoplasm from which the merozoites are formed.

The present study does not clarify the possible sequence of events for the first two days after inoculation. It has been suggested (Garnham, 1951, and others) that there may be a primary pre-erythrocytic cyle followed by a second cycle, perhaps both taking place in the liver, or perhaps the primary cycle may occur elsewhere. There is no certainty that all stages seen in our specimens were of one cycle or the other, if two cycles exist.

It is possible that definite ages cannot be ascribed to the various parasites with certainty until such time as an experiment is performed wherein each volunteer is given only one inoculation and liver samples are taken at a specified day or days later. This should help clarify also the question of the number of cycles in the pre-erythrocytic phase.

The failure to find exo-erythrocytic forms in material derived from the first 13 cases probably can be explained on the basis of sporozoite dosage, scarcity and poor quality of liver tissue, and inoculation-biopsy schedules. Patient 14, where parasites were found, received enormously larger sporozoite doses than any of the previous 13 (table 2). Even at this level of inoculation, only about 125 parasites were located in a fairly large tissue sample. Tissue samples in all but one (No. 11) of the first 13 cases were minute when compared to that obtained from No. 14. In addition to the small size of the tissue sample taken with the peritoneoscope forceps, the quality of the tissue derived left much to be desired. The specimens were taken necessarily from the inferior border of the liver, which is perhaps more fibrous and more capsular than any other portion of the liver. As a result, sections prepared from this material often yielded only a very small sample of actual liver parenchyma. As is shown in tables 1 and 2, patient 14 (the successful experiment) received a total of 8,516 bites and 1,403 glands from

mosquitoes which were 86.8 per cent infected. By a conservative estimate this should equal 20,000 infective bites. This is believed to be the largest inoculation of malarial parasites ever given to a human.

It is of interest that the resulting course of the infection in this case appeared to be normal.

SUMMARY AND CONCLUSIONS

Liver tissue was taken from 14 patients after massive sporozoite inoculations with a Panama strain of *Plasmodium falciparum*. The tissue was fixed in Carnoy's fluid, serially sectioned, and stained with a colophonium-Giemsa method.

Exo-erythrocytic parasites were found in the material derived from the patient receiving the heaviest inoculations. This patient had been inoculated on four successive days with a total of 8,516 mosquito bites and 1,403 salivary glands intravenously. The mosquitoes used averaged 86.8 per cent infected. Biopsy was done at laparotomy three to six days after inoculation. A total of about 125 parasites was found. The characteristics of these parasites indicate that they are part of the exo-erythrocytic phase of malaria. They are similar to those found by Shortt and his associates for this species of malaria.

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Explanation of Plates

All illustrations are unretouched photomicrographs at a magnification of about 900 ×. Unless otherwise indicated all figures are from sections cut at six microns and stained with Shortt and Cooper's (1948) colophonium-Giemsa stain.

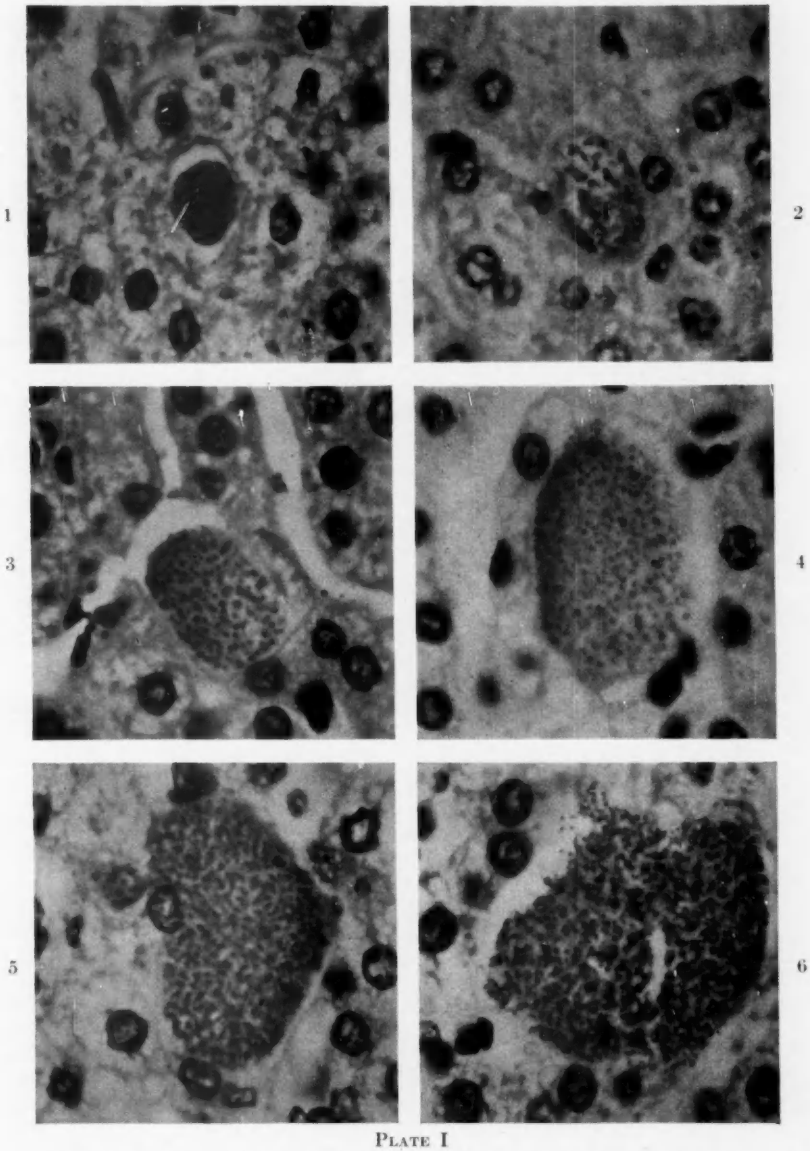


PLATE I

FIG. 1. This is one of the smallest parasites seen. The other section of this parasite is shown in figure 7.

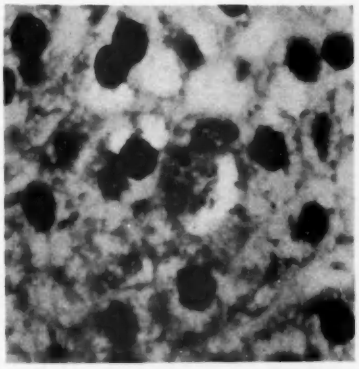
FIG. 2. A larger parasite than that shown in figure 1. Sections cut at two microns and stained with Delafield's hematoxylin.

FIG. 3. A larger stage than that in preceding figures.

FIG. 4. A still larger stage with nuclei and cytoplasm more condensed on the left side. Note that although sinusoids may be clearly seen on each side of the parasite, the parasite is not in contact with these spaces.

FIG. 5. A parasite approaching maturity, with vacuolization cutting the cytoplasm into cords and islands (Shortt's "pseudocytomeres"). Note the growth of the parasite around the unchanged nucleus of the hepatic epithelium.

FIG. 6. A mature schizont. Note the cords and islands in the parasite and the formation of merozoites, especially at the top of the parasite.



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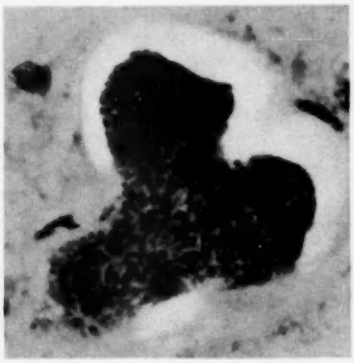

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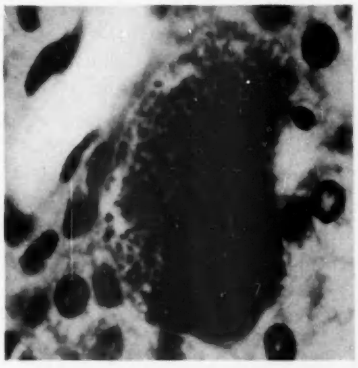

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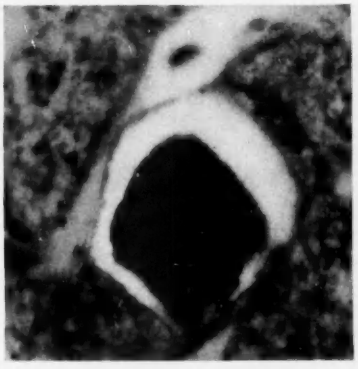

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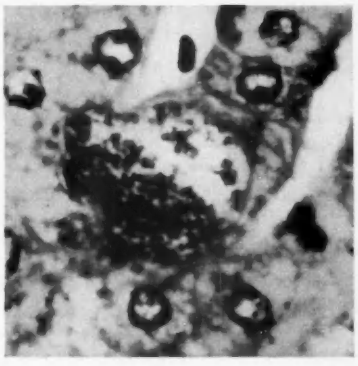

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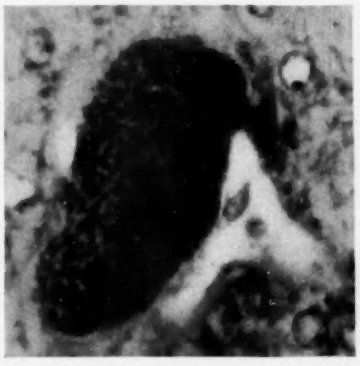

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PLATE II

FIG. 7. A small parasite, see also figure 1. Note a portion of the host-cell nucleus at the upper edge of the parasite.

FIG. 8. A schizont, mature or nearly so, showing lobe formation. Sections cut at three microns.

FIG. 9. A schizont showing merozoite formation. The space to the upper left is a blood vessel.

FIG. 10. A shrunken parasite. Note distention of the parasitized hepatic cell, relation to sinusoid and growth of the parasite around the hepatic cell nucleus.

FIG. 11. Note relation of the parasite to the liver cord and sinusoids. Sectioned at two microns and stained with hematoxylin and eosin.

FIG. 12. A schizont showing relation to a sinusoid, and growth into adjacent tissue, toward the right.

THE EFFECT OF ANTIBIOTICS ON EXPERIMENTAL MALARIA (*PLASMODIUM CATHEMERIUM* AND *PLASMODIUM BERGHEI*)[1]

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Early experiences with antibiotics and malaria led to an impression that an effective antimalarial was hardly likely to be found among these therapeutic agents. Of those which showed a plasmocidal effect, Taliaferro *et al.* (1944) found tyrothricin and one of its components, gramicidin, effective near the toxic range against *Plasmodium gallinaceum*, while Hewitt *et al.* (1950) reported clavicin to have an antimalarial activity close to the toxic range in birds. Aureomycin proved to be effective against blood-induced infections of *P. gallinaceum* and to act as a causal prophylactic against the same parasite in chicks (Coatney *et al.*, 1949). When used in human infections with *vivax* malaria, aureomycin had some definite prophylactic effect, although it had a very poor activity against patent blood infections and did not prevent relapses (Cooper *et al.*, 1950). Since the initiation of our experiments, Ruiz-Sanchez and his group in Mexico (1951, 1952) have reported the use of chloramphenicol, terramycin, and aureomycin as therapeutic agents in human malaria with some success. Because of the evidence of activity among the antibiotics, we decided to screen a number of them, especially those produced by various species of *Streptomyces* for possible antimalarial properties.

MATERIALS AND METHODS

We have used two parasites for the screening of the antibiotics tested, *Plasmodium cathemerium* (3H2-1 strain) in canaries and *Plasmodium berghei* (KBG 173 strain) in white mice. Of the drugs used, dihydrostreptomycin and neomycin were injected subcutaneously twice daily. The remainder of the drugs were administered perorally twice daily in bird experiments and as a drug-diet mixture to the mice used in *P. berghei* experiments.

Suppressive tests with *P. cathemerium* were carried out in accordance with procedures described earlier by Gingrich (Wiselogle, 1946) and Gingrich and Schoch (1947). Canaries weighing 15–20 grams were inoculated with 0.5 million parasites intravenously. The first dose of the drug was administered soon after inoculation and treatment continued for a total of 4.5 days. Comparison of the antibiotics with quinine was made on the basis of minimum effective doses as determined by parasite counts on the 5th day after inoculation. Short-term curative tests, found adequate for test of curative effect with this strain and inoculum of *P. cathemerium* (Gingrich *et al.*, 1947) were performed by the same methods employed for finding the suppressive effect except that maximum

[1] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

tolerated doses rather than minimum effective doses of the drugs were administered. Daily blood smears were taken until the birds became positive. Prophylactic tests with *P. cathemerium* involved inoculating the birds intravenously with sporozoites from one infected *Culex pipiens* per bird. Treatment was initiated the day before inoculation and continued to cover a period of 4 days or, in some experiments, for 10 and 20 days. Blood examinations were made daily beginning the 5th day after inoculation and continued until parasites appeared in the blood or for a total of 60 days. Birds remaining negative at the end of this time were subsequently tested for absence of the parasite by two subinoculations of their blood into clean birds and a final challenge of their immunity by an intravenous inoculation of 10 million parasites (Gingrich *et al.*, 1947).

Suppressive tests using *P. berghei* as the test organism were carried out by intravenous inoculation of white mice weighing 15–20 grams with 1 million parasites. Earlier experiments for standardizing the inoculum had indicated that with the intravenous inoculation of 1 million parasites, 100 per cent of the animals are infected and the parasitemia rises through the fifth day after inoculation, then falls and rises irregularly until death. For this reason, the drugs were administered in the diet for 5 days following inoculation and blood smears made on the 4th and 5th days after inoculation while the parasite count was still rising. A comparison between quinine and the antibiotics was obtained on the basis of the minimum effective doses of the drugs resulting in nearly equivalent parasitemias (as measured by the average number of parasites per 1000 erythrocytes for 5 mice).

RESULTS

Suppressive Tests

The results of suppressive tests with the antibiotics using *P. cathemerium* in canaries are shown in Table 1. Of those tested, aureomycin[2] showed the greatest effect with a quinine equivalent of 1. Terramycin[3] had a similar suppressive effect with doses approximately six times as great, and chloramphenicol[4] showed a very slight effect at close to toxic levels, with a quinine equivalent of 0.004. Other antibiotics tested, including dihydrostreptomycin, neomycin, rimocidin[3], thiolutin[3], and two Brucella-active lyophilized broths[3] had no suppressive effect in tolerated doses.

The results when four of the same antibiotics were tested against *P. berghei* in white mice are given in Table 2. Aureomycin and terramycin showed a suppressive effect similar to that found for *P. cathemerium* in the canary, while the

[2] Aureomycin hydrochloride used in these experiments was supplied through the kindness of Dr. H. R. Cox of Lederle Laboratories.

[3] Terramycin hydrochloride, rimocidin, thiolutin, and the two Brucella-active lyophilized broths were supplied through the kindness of Dr. E. A. Weyer of Charles Pfizer and Company.

[4] Chloramphenicol was supplied through the kindness of Dr. P. E. Thompson of Parke Davis and Company.

quinine equivalent of chloramphenicol was greater in infections with *P. berghei* (0.17) in the white mouse than in birds harboring *P. cathemerium* infections. Whether this greater suppressive effect was a result of the method of administration of the drug, better absorption of the drug in mice than in birds, or greater susceptibility of the parasite to chloramphenicol, remains to be shown. Neomycin had no suppressive effect on *P. berghei* infections.

Curative Tests

Aureomycin and terramycin, the most promising of the antibiotics, were tested as curative drugs on *P. cathemerium* infections in canaries. No cure resulted from either of these drugs when used at maximum tolerated doses singly and in combination with quinine (see Table 3).

TABLE 1

Results of suppressive tests with Plasmodium cathemerium *in canaries*

DRUG	QUININE EQUIVALENT
Aureomycin	1
Terramycin	0.167
Chloramphenicol	0.004
Dihydrostreptomycin	inactive at 300 mgm./Kg. daily
Neomycin	inactive at 300 mgm./Kg. daily
Rimocidin	inactive at 300 mgm./Kg. daily
Thiolutin	inactive at 4 mgm./Kg. daily
Brucella-active lyophilized broths	inactive at 1000 mgm./Kg. daily

TABLE 2

Results of suppressive tests with Plasmodium berghei *in the mouse*

DRUG	QUININE EQUIVALENT
Aureomycin	0.9
Terramycin	0.16
Chloramphenicol	0.17
Neomycin	inactive at 280 mgm./Kg. daily

* Reference dose of quinine base: 77 mgm./Kg. daily (average)

Prophylactic Tests

As shown by Table 4, the same three antibiotics having a suppressive effect, also caused a delay in the appearance of parasitemia in canaries inoculated with sporozoites of *P. cathemerium*. When administered for 4 days, chloramphenicol, aureomycin, and terramycin delayed the appearance of parasitemia for from two to twelve days beyond the five days required for parasites to appear in the blood of controls. Dihydrostreptomycin and neomycin had no effect on the progress of the infection. Complete prophylaxis did not occur except when the antibiotics were administered for 10 days or more. Half of the birds (3 out of 6) were af-

forded complete protection by aureomycin at 500 to 600 mg. of base per kilo daily for 10 days, while parasitemia was delayed in the remainder of the birds at these dosages for an average of 18 and 26 days. When the same dosage of aureomycin was continued for 20 days, all of the birds were completely protected. Terramycin completely protected two out of three birds at a dosage of 2.25 grams of base per kilo daily for 10 days, while the one remaining bird in this

TABLE 3

Results of short-term curative tests with Plasmodium cathemerium *in the canary*

DRUG	DAILY DOSE MGM. BASE/KG.	DURATION OF TREATMENT	CURES/NO. BIRDS
Aureomycin	500	4.5 days	0/4
Terramycin	2000–2500	4.5 days	0/6
Aureomycin + Quinine	500 / 100	4.5 days	0/4
Terramycin + Quinine	2000 / 100	4.5 days	0/2

TABLE 4

Results of prophylactic tests with Plasmodium cathemerium *in canaries*

DRUG	DAILY DOSE MGM. BASE/KG.	DURATION OF TREATMENT	AVERAGE NUMBER OF DAYS DELAY OF PARASITEMIA	NUMBER OF BIRDS COMPLETELY PROTECTED/TOTAL
Dihydrostreptomycin	300	4 days	0	0/4
Neomycin	160	4 days	0	0/1
	75	4 days	0	0/4
Chloramphenicol	3500	4 days	2	0/2
Aureomycin	500–600	4 days	11.2	0/5
	500	10 days	>18	2/4
	600	10 days	>26	1/2
	500	20 days	—	3/3*
Terramycin	1000–2500	4 days	11.7	0/6
	2250	10 days	>15	2/3
Controls	—	—	0	0/8

* A fourth bird belonging to this group was still free of parasites when it died on the 48th day after inoculation.

group was free of demonstrable blood parasites for 15 days beyond the time parasites appeared in the controls.

SUMMARY AND CONCLUSIONS

Certain antibiotics derived from various types of *Streptomyces* possess definite antimalarial properties. Against *Plasmodium cathemerium* in the canary, aureomycin and terramycin have marked prophylactic effect and even afford complete protection in some instances when administered for 10 days or longer. Aureomycin has a suppressive effect equal to that of quinine, and terramycin a relatively poor suppressive effect against the blood parasites. Chloramphenicol

has a meager prophylactic and suppressive effect only in maximum tolerated doses. None of these drugs proved to be curative, either alone or in combination with quinine. Dihydrostreptomycin, neomycin, rimocidin, thiolutin, and two Brucella-active lyophilized broths were totally ineffective.

The same three antibiotics, aureomycin, terramycin, and chloramphenicol, show a suppressive effect in *P. berghei* infections in the white mouse. Aureomycin is again nearly equivalent to quinine, while terramycin and chloramphenicol both have rather low quinine equivalents of 0.16 and 0.17 respectively. Neomycin had no suppressive effect on *P. berghei* infections.

The marked prophylactic effect of certain of these antibiotics, confirming previous reports of such activity by others indicates further search for more effective preparations in this group. To our knowledge, causal prophylaxis is demonstrated by only two other compounds against *P. cathemerium* infections—metachloridine (Wiselogle, 1946) and Endochin (unpublished observations). As this type of antimalarial activity may be interpreted as bearing chiefly on the exo-erythrocytic forms of the parasite, there is a possibility that among the antibiotics or a combination of one of them with another antimalarial will be found a less toxic curative agent for *vivax* malaria.

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BLOOD SUGAR STUDIES ON THE WHITE RAT INFECTED WITH *PLASMODIUM BERGHEI*

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INTRODUCTION

Although physiological research in the field of mammalian malaria has been extensive, it has dealt so far primarily with the malaria parasite itself, its metabolism, and the various conditions that influence its development. Not much attention has been directed toward studying the influence of the parasite on the physiology of the host, in particular its carbohydrate metabolism. Except for the contributions of Christophers and Fulton (1938), who investigated the effect on the blood sugar of monkeys infected with *Plasmodium knowlesi*, little is known regarding this phase of the malarial infection. *Plasmodium berghei* lends itself admirably for such a study since it is a mammalian species easily available and well controlled in the laboratory. The present investigation deals with the effect of this parasite on the blood sugar of the white rat, *Rattus rattus*.

MATERIALS AND METHODS

In this investigation the KBG 173 strain of *Plasmodium berghei* was used. Female white rats were inoculated intravenously with 0.15 cc. of a 1/100 dilution of heavily parasitized blood. The blood samples were obtained by cardiac puncture using ether anesthesia and heparin as the anticoagulant. The quantitative glucose determinations were made according to the method of Hagedorn and Jensen (1923). The glucose levels were determined at the beginning of patency and at short intervals during the patent period of the infection. The per cent of parasitized red cells was determined for each specimen studied. The parasitemia was expressed as the per cent of parasitized red cells rather than as the per cent parasite count because of the common occurrence of multiple infections. The blood smears were stained with Giemsa.

Most determinations were made on whole blood but additional separate determinations were made on serum and packed cells. The procedure followed in determining the sugar content of serum and packed cells was essentially that used for whole blood except that iodoacetic acid to produce a final concentration of M/1000 was added to the blood samples immediately after withdrawal and the specimens were then centrifuged at 1500 r.p.m. for 15 minutes. Following centrifugation the serum was carefully removed with a capillary pipette. The iodoacetic acid was added to inhibit glucose consumption during the time between withdrawal of the blood and the actual precipitation of the protein. Fulton (1939) has shown that glucose is an important nutrient of the malaria parasite and therefore the above procedure appeared necessary in order to hold the glucose content at the level of freshly drawn blood. This step was omitted in the determinations made on whole blood because the actual glucose determination followed immediately after the blood was obtained.

Control determinations were also carried out on material from uninfected white rats of the same age and sex as the infected specimens; the technique followed was the same as explained above except that the iodoacetic acid was omitted.

It is realized that the determination of the glucose content of packed cells according to the method described had its limitations because complete packing of the cells was not obtained and an undetermined, though probably small, admixture of serum remained in the analyzed sample. Complete packing would have prevented measuring the volume of the sample by means of the blood sugar pipettes. However, even with this limitation, it was preferred to determine the sugar content of the red cells directly rather than by calculation.

EXPERIMENTAL RESULTS AND DISCUSSION

The normal glucose content of whole rat blood was found to be 96 ± 3.7 mg. per cent. This level varied little during the course of the *P. berghei* infection except when the parasitemia rose above 41 per cent; the glucose content of the

TABLE 1

Glucose content of whole blood in white rats infected with P. berghei

NUMBER OF DETERMINATIONS	PER CENT PARASITEMIA	MG. PER CENT GLUCOSE
25	0–10	103 ± 6.9
14	11–20	89 ± 6.7
13	21–30	88 ± 8.9
16	31–40	89 ± 7.5
13	41–50	74 ± 4.1
11	51–over	68 ± 6.5
Controls (38)	Uninfected	96 ± 3.7

highly parasitized blood ranged from 74 ± 4.1 mg. per cent to 68 ± 6.5 mg. per cent. When the parasitemia was lower the values ranged from 103 ± 6.9 to 89 ± 7.5 mg. per cent (Table 1).

Christophers and Fulton (1938) reported a similar drop in the blood sugar of monkeys infected with *P. knowlesi*; from a normal level of 90 to 120 mg. per cent the glucose content decreased to 78 mg. per cent. As in these studies the lowering of the blood sugar in the monkey was not as marked when the parasitemia was less intense. A marked lowering of the blood sugar has also been observed in trypanosome infections where it appears to be due to a metabolic disturbance of the carbohydrate metabolism of the host rather than being directly related to the sugar consumption of the parasites (von Brand, 1951).

The glucose content of the packed cells remained fairly constant throughout the course of the infection although the values obtained were higher when compared with the normal level of 60 ± 3.5 mg. per cent; the level ranged from 72 ± 7.4 mg. per cent when the parasitemia was 10 per cent or lower to 103 ± 7.7 mg. per cent when the count was 30 per cent. When the parasitemia was higher than 30 per cent the glucose content ranged from 79 ± 4.8 to 88 ± 7.4 mg. per cent. The serum glucose level however was quite sensitive to an increase

in the parasitemia; the normal level of 155 ± 4.9 mg. per cent dropped to 55 ± 4.9 per cent when the parasitemia was 51 per cent and higher. Significant decreases were also observed when the parasitemia was lower i.e., 86 ± 10.3 mg. per cent at 31 to 40 per cent and 82 ± 11.2 mg. per cent at 41 to 50 per cent (Table 2).

This disturbance in the serum-erythrocyte glucose ratio is comparable to the changes observed by various investigators in the ionic composition of plasma and red cells in both human and simian malaria. Zwemer *et al.* (1940) observed an increase in the plasma potassium in human malaria and it was noted that the maximum concentration occurred when there was the greatest number of segmenting parasites. These investigators also noted an increase in the serum potassium in monkeys infected with *P. knowlesi*. McKee *et al.* (1946) observed an increase in the plasma and red cell sodium and potassium and plasma chloride but a slight decrease in the red cell chloride and a distinct decrease in the inorganic phosphate in both plasma and red cells. Overman *et al.* (1949) attributed these changes in the concentrations of the red cell potassium and sodium during

TABLE 2

Glucose content of serum and packed cells in white rats infected with P. berghei

NUMBER OF DETERMINATIONS	PER CENT PARASITEMIA	MG. PER CENT GLUCOSE IN SERUM	MG. PER CENT GLUCOSE IN PACKED CELLS
4	0–10	114 ± 12.1	72 ± 7.4
3	11–20	125 ± 4.1	85 ± 6.7
4	21–30	109 ± 22.8	103 ± 7.7
6	31–40	86 ± 10.3	82 ± 5.0
11	41–50	82 ± 11.2	88 ± 7.4
8	51–over	55 ± 4.9	79 ± 4.8
Controls (34)	Uninfected	155 ± 4.9	60 ± 3.5

a malarial infection to abnormal cellular permeability brought about as a result of the infection. A similar mechanism may be one of the contributing factors for the changes reported here although definite conclusions can only be reached after further study.

SUMMARY

1. The blood sugar level of white rats infected with *Plasmodium berghei* decreased to 68 ± 6.5 mg. per cent from a normal value of 96 ± 3.7 mg. per cent when the parasitemia was 51 per cent and higher.

2. The glucose content of the packed cells remained fairly constant during the course of the infection although the values obtained were higher when compared with the normal content of 60 ± 3.5 mg. per cent. The level ranged from 72 ± 7.4 to 103 ± 7.7 mg. per cent.

3. The normal serum glucose showed a marked drop to 55 ± 4.9 mg. per cent from the normal level of 155 ± 4.9 mg. per cent when the parasitemia was 51 per cent and higher.

ACKNOWLEDGMENTS

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CHLORAMPHENICOL IN MALARIA

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We recently reported promising results obtained with chloramphenicol in the treatment of two cases of malaria (Ruiz-Sánchez *et al.* 1951b). We present in this communication the results of 15 more cases treated during the fall of 1951.

In spite of the important sanitary work done by the Federal Public Health Service, malaria still occurs rather frequently in Guadalajara. This city of 400,000 inhabitants is situated about 5,000 feet above sea level and enjoys a mild temperate climate with little seasonal variation. *Plasmodium vivax* is the parasite most frequently found, although *P. falciparum* is not rare. The most important transmitting agents are *Anopheles quadrimaculatus* and *Anopheles albimanus*, whose breeding places are now limited to the springs and streams found around the city in the rainy season.

CASES

All the patients were admitted to the Civil Hospital of Guadalajara. There were nine men and six women, and their ages ranged from 13 to 60 years. Ten had typical paroxysms daily and the other five had them every other day. All had moderate enlargement of the spleen, and only five had enlarged livers. The duration of illness varied from 6 days in the shortest case to 32 days in the longest. *P. vivax* was found in 12 cases, *P. vivax* and *P. falciparum* together in 2 cases, and *P. falciparum* alone, in 1 case. In all cases sexual and asexual forms were seen. Red blood cells ranged from 1,390,000 to 4,300,000; white blood cells from 3,800 to 10,400, and hemoglobin from 2.5 grm. to 12.5 grm.

We used capsules of 250 mg. of chloramphenicol given to us by Dr. E. H. Payne of Parke, Davis and Co., Detroit, Michigan. The total daily dosage was from 50 to 75 mg. per kilo of body weight, given in three equal doses, one every eight hours. Treatment lasted eight days in 13 patients, and six and ten days respectively in the other two.

RESULTS

In all cases the infection was controlled within a few days. Symptoms disappeared, and the parasites from the circulating blood except for the sexual forms of *P. falciparum*. After treatment was started, paroxysms occurred from 0 to 5 times, with an average of 3.5. In 9 cases paroxysms became gradually and progressively less intense before ceasing entirely (Figs. 1 and 2). In these cases, toward the end of treatment, there was ordinarily no chill, or if present it was very mild.

The parasites remained in the peripheral circulation from 8 to 10 days after

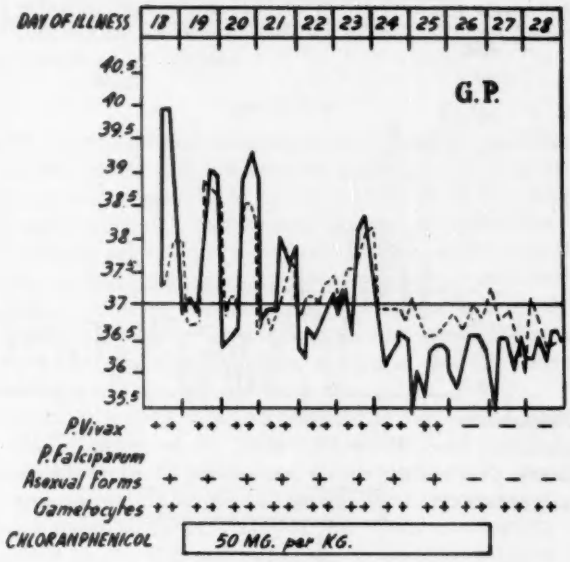

FIG. 1

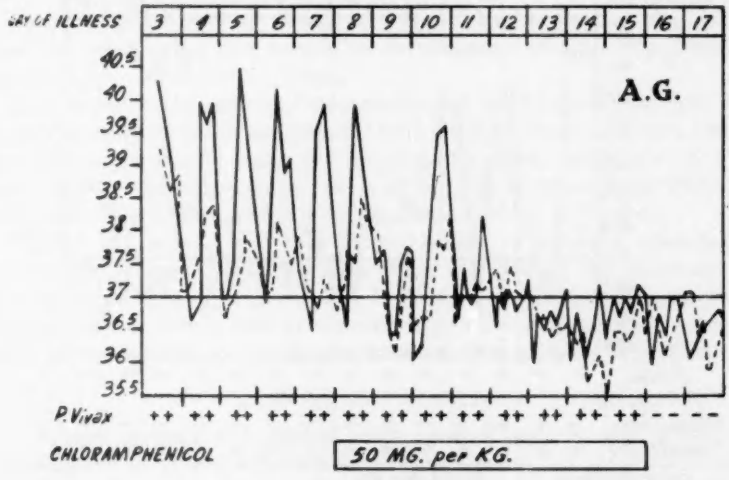

FIG. 2

treatment was started, with an average of 9 days and their disappearance was gradual. The gametocytes of *P. falciparum* persisted in the blood throughout the whole period of observation (Figs. 3 and 4).

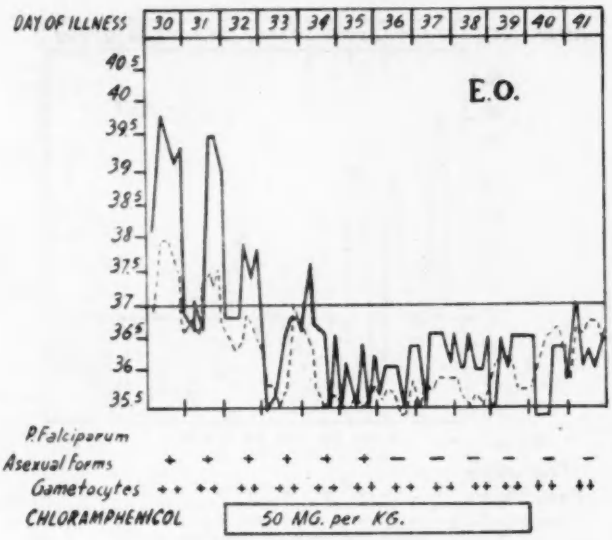

FIG. 3

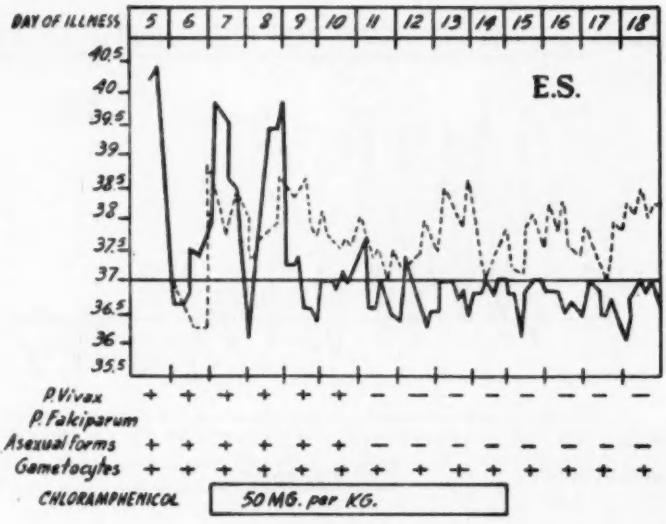

FIG. 4

The general condition of the patients improved at the same time; red cells and hemoglobin increased, the spleen became normal and asthenia disappeared.

RELAPSES

Our cases were discharged from 3 months to 6 weeks ago. As we write this report, there have been no relapses.

DISCUSSION

We have the impression that chloramphenicol definitely modifies the course of malaria and this report thus confirms the results published by us previously (*loc. cit.*). This view is supported by the disappearance of the symptoms early in the course of the treatment regardless of the type of Plasmodium; the clinical picture of the disease prior to the treatment; the favorable course of the illness once treatment was instituted, with more or less uniform results in the 17 cases; and the gradual but definite disappearance of the parasites from the peripheral circulation during treatment. Nevertheless the therapeutic effect of chloramphenicol is very slow and clearly inferior to that of the other commonly used antimalarial drugs.

It is of interest to note that, as we reported in the case of aureomycin and terramycin (Ruiz-Sánchez *et al.*, 1951, 1951a, 1952) chloramphenicol has no action on the gametes of *P. falciparum*, which persist in the peripheral blood after the disappearance of symptoms and conclusion of treatment. In the two cases of double infection (Figs. 1 and 4), the sexual and asexual forms of *P. vivax* disappeared on the fifth and eighth day of administration of the drug, as well as the asexual forms of *P. falciparum*, but the gametes of *P. falciparum* persisted during the whole time of stay in hospital.

We do not know that the dosage used in each case constitutes the optimum dose. We believe that this, as well as the minimum effective dose, should be determined by further observations.

With respect to the action of chloramphenicol on the pre-erythrocytic forms of the Plasmodium, described by Shortt *et al.* (1948) in human malaria, and consequently concerning its value as a prophylactic agent, Imboden *et al.* (1950) have recently reported complete failure of the drug in two subjects who voluntarily submitted to inoculation with the Chesson strain of *P. vivax*.

Whether our patients have been definitely cured, or will suffer relapses in the future is not known. Continued observation will give us this information. In this regard we have the experience of Imboden *et al.* (1950) who reported that chloramphenicol showed itself to be useless in preventing relapses, which occurred at the same intervals as in the experimental cases treated solely with chloroquine.

SUMMARY AND CONCLUSIONS

1. Fifteen patients with typical acute cases of malaria were treated with chloramphenicol by the oral route. In 13 cases *Plasmodium vivax* was found; in one *Plasmodium falciparum* was found alone, and two were mixed infections.

2. The dose varied from 50 mg. to 75 mg. per kilogram of body weight per day. Duration of treatment was 8 days in 13 cases, and 6 days and 10 days respectively in the other two. Further observations will be necessary to determine the minimum effective dose.

3. Results were positive in all cases. The number of attacks which occurred after beginning of treatment varied from 0 to 5 with an average of 3.4. The parasites disappeared between the second and tenth day of treatment in the cases with *P. vivax*. In the infections with *P. falciparum* only the asexual forms disappeared during this period; the sexual forms persisted and the drug apparently had no action on them.

4. In the subsequent short period of observation there were no relapses, but we cannot say that these patients have been definitely cured.

5. The therapeutic value of chloramphenicol in malaria is markedly inferior to that of other drugs in common use.

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MALARIA IN THE LEEWARD AND WINDWARD ISLANDS, BRITISH WEST INDIES[1]

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Field observations on the epidemiology of malaria have appeared in the literature in relation to the larger Caribbean Islands which form the Greater Antilles. Thus, Boyd & Aris (1929) reported on a malaria survey of Jamaica; Earle (1930) reviewed the epidemiology of malaria in Puerto Rico; Carr & Hill (1942) summarized survey findings in Cuba; while Paul and Bellerive (1947) reported on a reconnaissance of the Republic of Haiti.

In the British Units of the Lesser Antilles results have been reported by de Verteuil (1933) and Downs *et al.* (1943) in Trinidad; by Earle (1936) on comprehensive surveys in St. Lucia; and by Root & Andrews (1938) in Grenada. Other references available emphasize in varying degree the malaria problems of several of these islands. Thus Nicholls (1912) studied the bionomics of anopheles in St. Lucia; Seagar (1928) reported on the Barbados malaria epidemic; Dyar (1928) referred to *A. tarsimaculatus* in the Lesser Antilles; Hoffman (1930) described the presence of *A. albimanus* in Nevis and Montserrat; Senevet (1936) referred briefly to the anopheles of Dominica; and Edwards & Box (1940) compiled detailed notes on the mosquitoes of Antigua.

For the greater number of the British island units, however, (with the four exceptions referred to above), systematic studies on malaria had not been undertaken prior to 1943. In order that this hiatus might be filled, and the necessary data made available on which to formulate malaria control policy in the Leeward and Windward Islands, a small Malaria Unit was established under the auspices of the Development & Welfare Organisation for the British West Indies. After initial training of a malariologist and an entomological and laboratory assistant by the Rockefeller Foundation Unit in Trinidad, field observations were commenced in March, 1943.

GENERAL CHARACTERISTICS

The Leeward and Windward Islands form a chain in the Caribbean Sea between Puerto Rico to the north and Trinidad to the south (Fig. 1). For administrative purposes they are divided into the Windward and Leeward Island Colonies, the former comprising the four largest—Grenada, St. Vincent, St. Lucia, and Dominica (together with smaller dependencies), and the latter consisting of the islands of Montserrat, Antigua, Nevis, St. Kitts (with lesser dependencies) which are dealt with in this report, and also the British Virgin Islands.

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They are all small, varying in area from 32.5 sq. miles in Montserrat to 305 in Dominica. With the exception of Antigua which is partly coral, the islands are of volcanic formation, each comprising a central range of mountains, running north to south and rising over 2,000 feet above sea level, from which numerous rugged spurs radiate to the coast where they generally terminate precipitously at the sea. There are no marginal plains of any extent, and it is rare

FIG. 1

that one can traverse a distance of half a mile from the coast without attaining an altitude of 100 feet.

The intervening narrow valleys are watered by small torrential rapids with rock-strewn sandy beds, which, during the relatively dry season and even in the intervals between heavy downpours, are subject to pooling and become partially blocked at their estuaries by sand bars created by wave action. This results in the formation of small coastal lagoons comparable to those found in Trinidad

(Downs *et al.*, 1943). Small mangrove swamps occur at the northern ends of St. Lucia and Dominica; on the southeast and northwest coasts of Grenada; at the southern end of St. Kitts; on the northwest coast of Nevis; and at several points along the irregular coastline of Antigua. A number of hillside seepages contribute to the problem of anopheles production in several of the islands.

Rainfall—In the more rugged islands of the Windwards Group, the rainfall (Table 1) is heavy, principally because of the influence of the northeast trade winds, the average annual precipitation over a 50-year period varying between 81.2 inches in Grenada and 114.4 inches in Dominica. Despite this, however, a comparatively "dry" season during February–May is usual, while a short "Indian Summer" or "Karem" during September–October is a common feature.

TABLE 1

Vital statistics, malaria morbidity and mortality (1942), and average annual rainfall, Leeward—Windward Is., B.W.I.

ISLAND	POPULATION		BIRTH RATE*	CRUDE DEATH RATE*	INFANT MORTALITY RATE†	MALARIA		AV. ANNUAL RAINFALL 50 YRS. (INS.)
	Census 1921	Estimated 1942				Morbidity rate	Mortality rate	
Grenada	66,302	87,805	28.8	19.1	131.0	12,098	108.7	81.2
St. Vincent	45,295	60,402	37.1	18.2	158.2	1,052	1.1	101.5
St. Lucia	51,503	66,360	35.4	20.2	117.6	14,526	330.2	87.2
Dominica	37,059	53,686	31.1	18.7	119.9	7,364	97.3	114.4
Montserrat	13,000	14,679	26.9	18.7	135.3	787	40.9	60.8
Antigua	29,592	37,127	39.2	15.8	97.4	2,803	47.2	42.8
Nevis	11,569	9,959				5,859	40.1	48.2
			30.8	18.9	140.4			
St. Kitts	22,415	29,367				0	0.0	54.6

* Per 1,000 Population.
† Per 1,000 Live Births.
‡ Per 100,000 Population.

The average monthly distribution for this group is typically illustrated as for Dominica in Fig. 2.

In the Leewards Group the annual rainfall is appreciably lower, although in general the monthly distribution (as exemplified in Fig. 2 for Antigua) follows closely that in the Windwards.

Climate—On the coast the climate is hot and humid, the temperature varying between an average maximum of 84°F. and minimum of 76°F., with an overall mean annual temperature of 79°F. and a relative humidity of 75 per cent. At higher altitudes, however, as at Sylvania in Dominica or the Grand Etangs in Grenada, the mean annual temperature is approximately 69°F.

The population for the various islands is shown in Tables 1 and 2. This is predominantly of Negro West Indian stock, but in St. Lucia and Grenada there are approximately 2,000 persons of East Indian origin—the descendants of indentured labor.

With few exceptions, e.g. in St. Vincent, Antigua and Nevis, the centers of

population are located on the coast, largely because of the importance of sea transport by small local craft for purposes of marketing in the principal towns, and because of the attraction of deep-sea fishing as a subsidiary occupation.

The bulk of the population however, is engaged in the production of sugar, sea-island cotton, citrus fruits, bananas, nutmegs and arrowroot. In the pre-

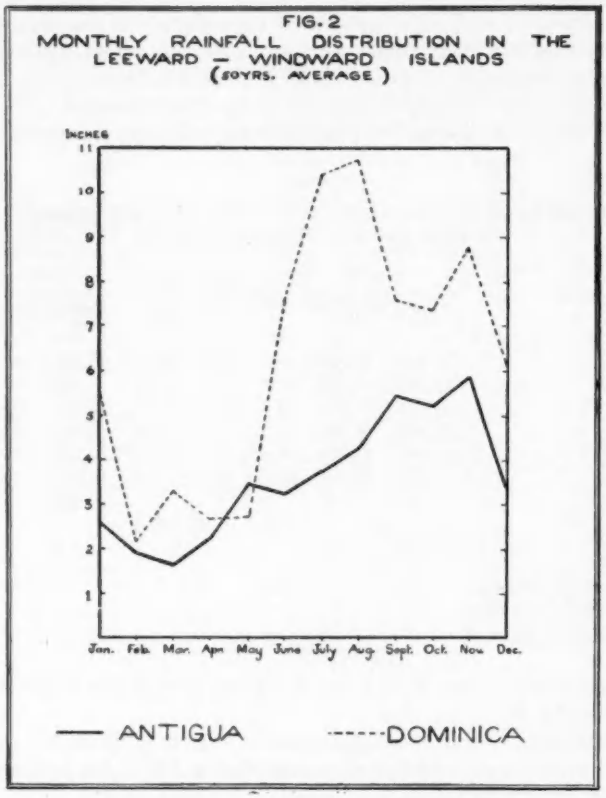

FIG. 2

dominantly sugar-producing islands of Antigua and St. Kitts the majority are dependent on estate and factory employment, but in the more rugged islands the tendency is toward peasant development.

Medical services—Each island is served by a small group of Government Medical Officers whose district populations vary between 5,000 and 9,000. There is usually a central hospital in the chief town, and in some instances there are one or more small cottage hospitals in the outlying areas.

Economic status—A brief outline of the economic state of the various islands

is shown in Table 2. During the two years immediately preceding World War II only St. Vincent managed to balance the annual budget without Imperial assistance while among the eight islands, Grenada and St. Vincent alone showed a favorable trade balance. As a result of this, administrative policy has tended to emphasize curative services at the expenses of measures in preventive medicine and public health.

Vital statistics—A brief summary of the vital statistics, malaria rates and average annual rainfall for each island is set out in Table 1. An overall low public health standard is illustrated by the high crude death rates and infant mortality rates.

TABLE 2

Economic status of the Leeward—Windward Is., B.W.I., 1939

ISLAND	AREA (SQ. MLS.)	POPULATION 1939	AVERAGE ANNUAL VALUE ($1000 B.W.I.)* OF			
			Revenue	Expenditure	Imports	Exports
Grenada	133	90,085	787	829	1,412	1,510
St. Vincent	150	59,683	490	473	954	1,011
St. Lucia	233	62,880	425	477	909	816
Dominica	305	50,426	282	333	494	372
Montserrat	32	13,541	130	179	284	198
Antigua	108	35,527	495	520	1,219	962
Nevis	50	9,463	472	574	1,448	1,278
St. Kitts	68	29,106				

* 1939 U.S. $1.00—B.W.I. $1.12.

METHODS OF SURVEY EMPLOYED

For evidence of splenomegaly, approximately half of the children in attendance at the large schools, and all those in the smaller schools were examined, lying recumbent on a convenient piece of school furniture, with knees and hips flexed. Bimanual palpation was made from the right side, and Boyd's (1930) method adopted for recording the results. The PDI spleens were assigned a value of 0.5.

Combined thick and thin blood smears were taken on all children with palpable enlargement of the spleen, and on 25 per cent of those with nonpalpable spleens. Since the blood samples were thus partly selected, adjusted parasite rates were computed by weighting the percentage of blood positives found in the normal-spleen groups as though all children palpated were examined for malaria parasites. Staining was made by bulk American Giemsa buffered at a pH of 7.2, and except for species identification on some thin smears, only thick smears were examined.

Anopheline larva collections were made and identified from all water deposits present within a one-mile radius of each center of population, while adult captures were made in houses, stables and on animal bait at a number of those villages where relatively high malaria endemicity was recorded.

RESULTS OF SURVEYS

1. Grenada: October–December 1945

Previous investigations and control: As a result of observations in 1929 by Root & Andrews (1938) and in 1933 by Earle (1936)—both under the auspices of the International Health Division, Rockefeller Foundation—a Paris green larvicidal control project was initiated in the northern more malarious part of the island at Sauters and Grenville but was discontinued after 2½ years. This was supplemented by major permanent works in 1935–36, but the maximum anticipated results were not realized. Under Cochrane and Newbold (1943), a second control programme based entirely on major engineering works on the west coast was initiated in 1942 under a 5-year Colonial Development and Welfare grant.

TABLE 3

Malaria morbidity rates, Leeward—Windward Is., 1933–1942*

YEAR	GRENADA	ST. VINCENT	ST. LUCIA	DOMINICA	MONTSERRAT	ANTIGUA	NEVIS	ST. KITTS
1933	6,542	1,731	3,582	7,384	0	7,743	1,051	0
1934	7,981	1,434	4,019	7,731	0	2,876	1,255	0
1935	14,423	2,710	13,624	6,985	0	3,175	1,332	0
1936	11,287	1,676	4,300	5,378	10	10,262	1,438	0
1937	10,686	650	8,996	8,821	20	8,378	846	0
1938	8,469	1,348	11,296	6,632	1,323	5,145	2,418	0
1939	9,098	857	15,885	7,194	1,259	3,018	956	0
1940	11,403	576	17,234	8,431	844	4,028	1,683	0
1941	12,805	913	10,659	8,621	1,924	1,289	3,276	0
1942	12,098	1,052	14,526	7,364	787	2,803	5,859	0
Average 1933–1942	10,526	1,272	11,743	7,431	522	4,833	2,029	0

* Per 100,000 Population.

Malaria prevalence in Grenada had always been reputed to be high. This was borne out by the fact that during the period 1933–1942 the average annual malaria morbididy and mortality rate was 10, 526 and 893 respectively (Tables 3 and 4).

Survey findings: Spleens and bloods—Basic data on spleen and blood examinations are presented in Tables 5, 6, 7 and 8. Of particular note was the low overall spleen rate of 9.1 per cent; the predominance of small spleens, and an adjusted parasite rate (8.7) nearly equal to the spleen rate. *Plasmodium falciparum* was most frequently found, *P. malariae* common, and *P. vivax* comparatively rare. In all areas of the island close positive correlation was found between the presence of splenomegaly and parasitism—the percentage of positive smears being in direct ratio to the degree of splenic enlargement.

Among 174 East Indian children included in the sample, the spleen rate (14.9) was statistically higher than that recorded (8.9) on 3,804 Negroes, but the significance of this finding was reduced by the fact that the majority of East Indians resided in the areas of higher malaria endemicity.

TABLE 4

Malaria mortality rates (per 100,000 population), Leeward—Windward Is., 1933—1942

YEAR	GRENADA	ST. VINCENT	ST. LUCIA	DOMINICA	MONTSERRAT	ANTIGUA	NEVIS	ST. KITTS
1933	53.2	19.1	70.4	113.3	0.0	75.3	35.2	0.0
1934	68.6	7.3	103.1	114.4	0.0	50.2	11.1	0.0
1935	95.3	22.6	140.2	98.0	0.0	14.1	67.4	0.0
1936	113.2	4.1	145.3	64.2	7.1	117.4	22.2	0.0
1937	86.5	8.3	138.2	111.1	14.3	140.6	11.1	0.0
1938	99.2	2.1	170.5	96.3	95.4	40.1	11.2	0.0
1939	62.3	5.1	309.2	92.4	44.1	23.7	0.0	0.0
1940	107.6	3.4	250.5	127.2	15.3	27.1	52.6	0.0
1941	98.6	3.3	331.8	95.7	231.3	24.1	20.6	0.0
1942	108.7	1.1	330.2	97.3	40.9	47.2	40.1	0.0
Average 1933–1942	89.3	7.6	198.9	100.9	44.8	55.5	27.1	0.0

TABLE 5

Results of malariometric surveys on school children, Leeward—Windward Is., 1943–1947

ISLAND	SPLEENS		BLOODS	
	No. examined	Spleen rate	Parasite rate (adjusted)	No. examined
Grenada	3,978	9.1	8.7	1,497
St. Vincent	1,774	0.7	0.0	457
St. Lucia	4,873	11.3	12.9	1,653
Dominica	4,310	9.8	10.4	1,483
Montserrat	100	0.0	0.0	25
Antigua	3,181	5.6	7.8	915
Nevis	1,692	1.9	1.9	352
St. Kitts	217	2.3	2.2	90
Total	20,125	7.7	8.5	6,472

TABLE 6

Degree of splenic enlargement, Leeward—Windward Is., 1943–1947

ISLAND	NO. ENLGD.	AVERAGE SPLEEN	AVERAGE ENLGD. SPLEEN	SPLEEN SIZES			
				PDI	1	2	3
Grenada	364	0.09	0.82	200	116	44	4
St. Vincent	12	0.01	0.91	6	4	2	0
St. Lucia	549	0.11	0.99	230	216	95	8
Dominica	425	0.09	0.96	186	167	65	7
Montserrat	0	0.00	0.00	0	0	0	0
Antigua	178	0.05	0.88	90	63	25	0
Nevis	33	0.01	0.70	24	7	2	0
St. Kitts	6	0.01	0.70	5	0	1	0
Total	1,567	0.07	0.94	741	573	234	19
Per cent of total enlarged				47.3	36.6	14.8	1.2

The survey findings indicated that the chief centers of malaria in 1945 were located in the middle two-thirds of the east coast, centered at Grenville (Fig. 3). This contrasted appreciably with the findings of Root & Andrews (1938) who in 1929 recorded their highest figures on the northeast and northern parts of the

TABLE 7

Association of positive parasite findings with degree of splenic enlargement, Leeward—Windward Is., 1943–1947

ISLAND	TOTAL WITH NORMAL SPLEEN		PDI		1		2		3		TOTAL WITH ENLARGED SPLEEN	
	No. examd.	Per cent pos.	No. examd.	Per cent pos.	No. examd.	Per cent pos.	No. examd.	Per cent pos.	No. examd.	Per cent pos.	No. examd.	Per cent pos.
Grenada	1141	5.9	197	31.0	112	40.0	44	54.5	3	66.6	356	33.0
St. Vincent	445	0.0	6	0.0	4	0.0	2	0.0	0	0.0	12	0.0
St. Lucia	1110	9.3	226	31.0	215	50.7	94	45.6	8	62.5	227	40.0
Dominica	1059	5.8	185	44.4	167	52.7	65	77.0	7	85.7	226	53.3
Montserrat	25	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Antigua	741	5.3	89	42.7	62	63.0	23	56.5	0	0.0	174	51.7
Nevis	319	0.6	24	58.3	7	57.1	2	100.0	0	0.0	33	60.5
St. Kitts	84	0.0	5	20.0	0	0.0	1	100.0	0	0.0	6	33.3
Total	4924	5.5	732	36.3	567	50.2	231	57.5	18	72.2	1548	45.0

TABLE 8

Relative frequency of the malaria parasite species, Leeward—Windward Is., 1943–1947

ISLAND	NO. OF BLOOD SMEARS EXAMD.	TOTAL POS.	PARASITE SPECIES: PER CENT OF TOTAL POSITIVES							
			Falciparum		Malariae		Vivax		Mixed	
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Grenada	1,497	200	134	67.0	60	30.0	5	2.5	1	0.5
St. Vincent	457	0	0	0.0	0	0.0	0	0.0	0	0.0
St. Lucia	1,653	331	188	56.8	126	38.1	10	3.0	7	2.1
Dominica	1,483	287	192	66.9	86	30.0	2	0.7	7	2.4
Montserrat	25	0	0	0.0	0	0.0	0	0.0	0	0.0
Antigua	915	129	80	68.0	49	32.0	0	0.0	0	0.0
Nevis	352	22	6	27.2	16	72.7	0	0.0	0	0.0
St. Kitts	90	2	1	50.0	1	50.0	0	0.0	0	0.0
Total	6,472	971	601	62.0	338	34.8	17	1.7	15	1.5

island. No effective control measures had been executed in these areas between the two observations.

Anopheles: All three species described by Root & Andrews (1938) were collected, viz. *A. aquasalis* (*tarsimaculatus*), *A. argyritarsis* and *A. pseudopunctipennis*, with the first predominating (Table 9).

A. pseudopunctipennis ranked second while *A. argyritarsis* comprised only 4

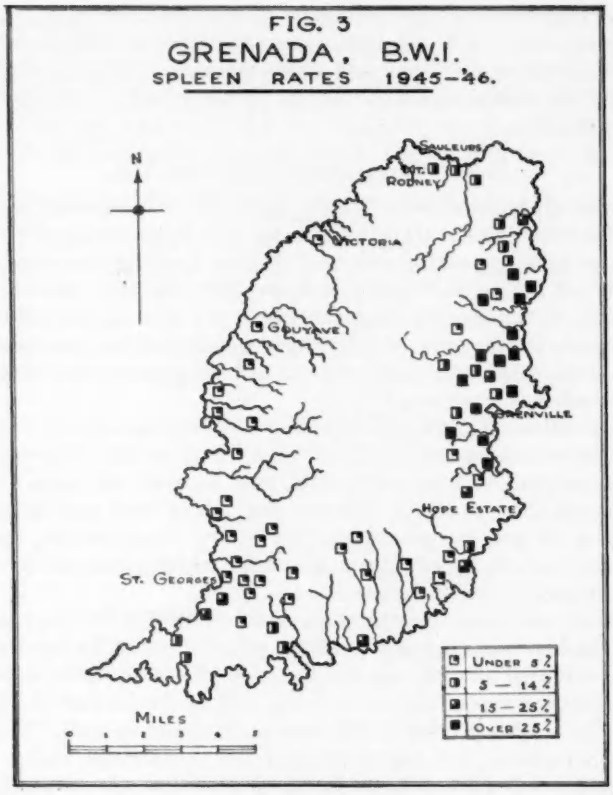

FIG. 3

TABLE 9

Results of Anopheles *larval collections, Leeward—Windward Is., 1943–1947*

ISLAND	SEASON	TOTAL LARVAE	AQUASALIS	ARGYRITARSIS	ALBIMANUS	PSEUDOPUNCTIPENNIS
Grenada	Oct.–Dec.	2488	2040	348	0	100
St. Vincent	Aug.–Sep.	1428	312	1115	0	0
St. Lucia	Apr.–July	1790	1445	245	0	0
Dominica	Mar.–May	2370	1327	1043	0	0
Montserrat	Jan.–Feb.	246	159	0	87	0
Antigua	Nov.–Feb.	2101	465	110	1526	0
Nevis	Feb.–Mar.	1353	226	0	1127	0
St. Kitts	November	56	56	0	0	0

per cent of the larvae collected. *A. aquasalis* was found in every known water deposit on the coast, with the exception of Lakes Antoine and Lavera in the north. The most important production foci were swamps and lagoons in the

vicinity of Pearl's Airport and Telescope on the northeast coast, and Morne Rouge Swamp south of St. Georges. It was collected in small numbers in the middle two-thirds of the west coast where permanent outlets had been constructed for the coastal lagoons under the Development and Welfare Scheme referred to above.

2. St. Vincent: August–September, 1944

In physical characteristics St. Vincent conforms to the general picture outlined for the other units of the Windward Islands, its central range being dominated by the 3,500-foot extinct volcano, Soufriere. It differs markedly, however, in that the soil is light and sandy, so that despite the heavy rainfall of 101.5 inches (Table 1), this island is singularly free from surface ponds and swamps.

Furthermore, the majority of the villages are situated on the hillsides or on the crests of the ridges overlooking the sea, and are therefore favorably located, from the standpoint of malaria.

Malaria prevalence: For the past 30 years St. Vincent has enjoyed a reputation of relative freedom from malaria. Local history suggests that following the last devastating eruption of Soufriere in 1902, large numbers of Caribs were rehabilitated on the coast at Arnos Vale and that among them malaria became so rampant that the area was evacuated. This locality proved, during the present survey, to be the most important *aquasalis* focus on the island and it continues, wisely, to be only sparsely populated.

The relative and recent unimportance of this disease in St. Vincent is illustrated by the low morbidity and mortality rates set out in Tables 3 and 4 respectively. Although all diagnosis was based on clinical evidence, considerable annual fluctuations appear to have occurred, ranging from a high of 1,508 cases and 12 deaths in 1935 to a low of 352 cases and 2 deaths in 1940. The majority of cases were reported from Buccament Bay and Cumberland Valley.

Survey findings: Spleens and bloods—In 15 localities only 12 children were found with splenomegaly (Tables 5 and 6), of whom 9 were among 46 residents on the banks of a lagoon at Buccament Bay. The other three small spleens were found at three settlements on the west coast. All the 457 blood smears examined were negative for malaria, but 2.4 per cent contained *Mansonella ozzardi.*

In the absence of schistosomiasis, or even of *Planorbis* snails, splenic enlargements in association with *A. aquasalis*—certainly at Buccament Bay—were probably due to malaria, although further observations are indicated.

Anopheles: Only two species were found, viz. *A. aquasalis* and *A. argyritarsis* —the latter predominating (Table 9). *A. aquasalis* occurred in 7 localities, the most important of which were Cumberland, Arnos Vale and Buccament Bay. *A. argyritarsis* was more widely distributed throughout the island. Senevet's (1936) report of *A. albimanus* in this island was not confirmed.

3. St. Lucia: April–July, 1943

Malaria prevalence: St. Lucia has always been reputed to be a highly malarious island. In the immediate past this has been due to extensive brackish swamps

on the outskirts of Vieux Fort at the southern end, and at Gros Islet in the north, which were wholly or partly reclaimed by the U. S. A. F. during 1941–42. A second important factor was the three comparatively large valleys under sugar cane cultivation where, because of the heavy nature of the clay soil, poorly graded field drains were maintained as semipermanent fixtures, and afforded extensive breeding grounds for *A. aquasalis*. Earle (1936) recorded high en-

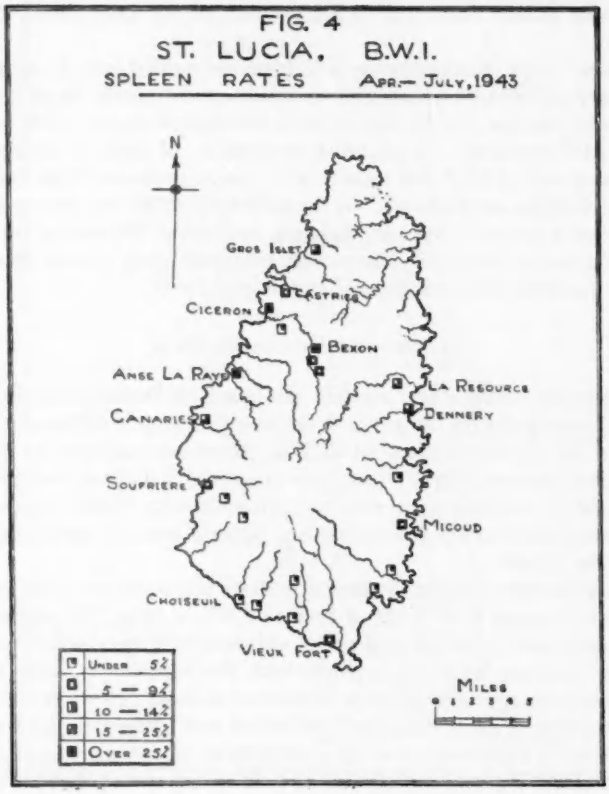

FIG. 4

demicity at Vieux Fort, Anse La Raye and Gros Islet, while Briercliffe (1941) recorded a spleen rate of 50 per cent at this latter village. The relatively high malaria morbidity and mortality as returned by medical practitioners during the period 1933–1942 are shown in Tables 3 and 4. As in the other islands, diagnosis was made almost exclusively on clinical history and examination.

Survey findings: Spleens and bloods—The general picture was comparable to that found in Grenada (Tables 5 to 8), with an overall spleen rate of 11.3 per

cent and a parasite rate adjusted at 12.9 per cent; small spleens; prevalence of *P. falciparum* and *P. malariae*; and the infrequent occurrence of *P. vivax*.

The existence of splenomegaly here also was closely associated with parasitism, both in regional distribution and in degree of enlargement. The areas with important spleen rates were located close to the sugar cane valleys, at Bexon (39.2%), Anse La Raya (38.3%), Ciceron (36.0%), Dennery (21.8%) and La Resource (20.0%), as illustrated in Fig. 4.

Mansonella ozzardi was found in 3.2 per cent of the 1,653 blood smears examined.

Anopheles: Larval searches made in 83 localities yielded only *A. aquasalis* and *A. argyritarsis* (Table 9). *A. argyritarsis* was most commonly found breeding in seepages and streams. Its highest altitude was approximately 1,200 feet above sea level, at Forrestierre. *A. aquasalis* occurred in all types of surface ground waters and at every inhabited locality with the exception of Forestierre in the hills above Castries, and Choiseul on the southwest coast. It was more common at or near sea level but it was recorded at an altitude of 750 feet at Terre Blanc. A small number of epiphytic plants were examined from several forest areas, but no anophelines of the subgenus *Kertezia* were found.

4. Dominica: March–May, 1944

In topography, climate and rainfall, the island of Dominica conforms with the general description for the group. A striking ethnological difference, however, is found on the northeast coast, at Salybia, where remnants of the Aboriginal Carib Tribe (approximately 500) continue to occupy a Native Reservation, but who, because of intermarriage with the predominantly Negro population are rapidly losing their native characteristics. Malaria was not prevalent in that section of the island.

Malaria prevalence: During the period 1933–42 the annual number of malaria cases reported ranged from 2,629 in 1936 to 4,579 in 1941. The morbidity and mortality rates, shown in Tables 3 and 4, indicated that the disease maintained a relatively constant level of prevalence with the exception of 1936 when the annual rainfall was appreciably below the 50-year average. Official returns showed that the majority of cases came from the towns and villages on the west coast, the small town of Portsmouth having a particularly notorious reputation.

Survey findings: Spleens and bloods—Details are set out in Tables 5–8 inclusive. As in St. Lucia, the spleen rate (9.8%) was exceeded by the adjusted parasite rate (10.4%). The general feature of small spleens; predominance of *P. falciparum* and *P. malariae* over *P. vivax*; and the close association between splenomegaly and parasitism were also here evident. Despite the low overall malariometric rates, however, high malaria spleen rates were recorded at Portsmouth (49.0%) and Calibishie (40.9%), with other areas of importance at Salisbury (26.6%), Mahaut (24.2%) and St. Joseph (21.2%) on the north and west coasts (Fig. 5).

Mansonella ozzardi occurred in 2.9 per cent of the blood smears examined, but

was limited to seven localities between St. Joseph and Portsmouth on the west coast. At Portsmouth, notorious for its periodic infestation by *Culicoides furens*, 13.3 per cent of 120 children were infected with this microfilaria.

Anopheles: Searches made in 111 localities yielded 2,370 *Anopheles* larvae (Table 9). The water types differed in no way from those recorded for St. Lucia, but the higher proportion of *argyritarsis* here was attributed to the more favor-

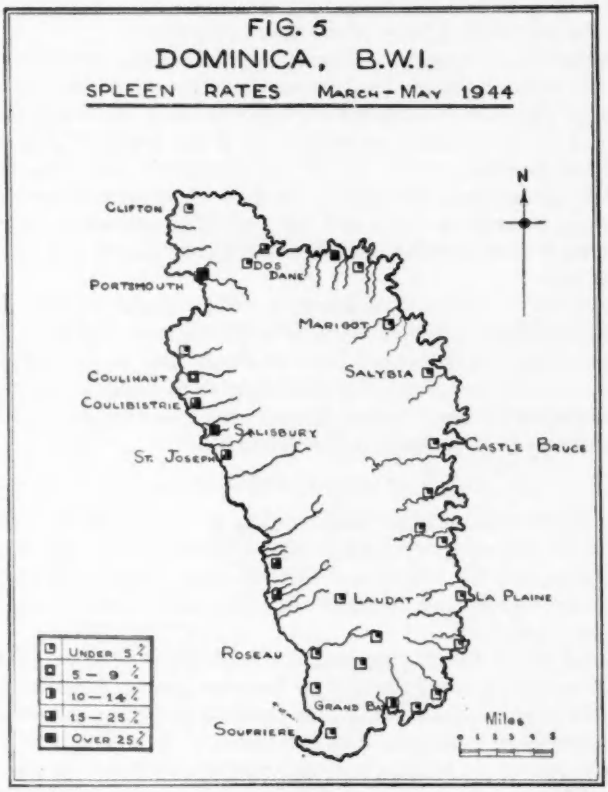

FIG. 5

able dry season during which collections were made. Both species were widely distributed throughout the coastal area, but the presence of *A. argyritarsis* in a wooden tub at Castle Comfort Estate, and in a roadside trench at Sylvania, approximately 1,800 feet above sea level was of particular interest. *A. albimanus*, reported from Dominica by Senevet (1936) was not collected. Examination of bromeliads at three forested areas (Bellevue, Chopin, Penrice and Branches) yielded negative results for the Subgenus *Kertezia*.

5. Montserrat

Topographically, this island resembles more closely the units of the Windwards Group, while the distribution of its population away from the coastal lagoons and swamps is even more pronounced than that noted in St. Vincent.

Malaria prevalence: Montserrat is generally regarded as free from malaria, and there is ample justification for this in Table 3, for all five cases of malaria diagnosed during 1933–37 had resided, during the critical part of the incubation period, in the neighboring island of Dominica or Antigua.

The presence of *albimanus* had already been established by Hoffman (1930), however, and a severe though localized epidemic of malaria occurred in 1938 at Farm Village. The disease continued for approximately four years, as shown in Tables 3 and 4, and extended to other parts of the island. *P. falciparum* was the important parasite species, and it was considered probable, though not proven, that this epidemic followed in the wake of immigrant gametocyte carriers returning home from Cuba and the Dominican Republic. Only sporadic cases have since been recorded, and therefore a comprehensive survey was not undertaken here.

Field observations: During 1949, however, while engaged on other duties, the writer observed heavy anopheles breeding in the mangrove swamp at Fox's Bay on the west coast, and in isolated pools at Carr's Bay to the north. Both *A. aquasalis* and *A. albimanus* were identified from these collections (Table 9), but no anopheles breeding was found at Farm during that visit, nor was there any evidence of recent malaria there (Tables 5 and 6).

6. Antigua: November 1943–February 1944.

Antigua differs from the other islands of the two groups in that it is divided into two parts—the north and eastern half which is relatively flat and of limestone formation, and the central and southern more rugged portion where the soil varies from a black loam to heavy clay. The highest peak is approximately 1,200 feet above sea level.

The rainfall is low, the 50-year annual average being 42.85 in. (Table 1 and Fig. 2), and severe periods of drought are frequent. Largely because of this, and in the absence of an adequate island-wide piped water supply, numerous shallow ponds form auxilliary sources for man and beast.

Malaria prevalence in Antigua has been comparatively low in the past, and characterized by periods of remission. Unfortunately, data on reported morbidity were not available for a sufficiently long period to determine accurately the true cyclical behavior of the disease. It was well established, however, that periods of prolonged drought such as occurred in 1939–1940 are followed by low malaria incidence (Table 3).

Here, a large proportion of cases were confirmed by laboratory diagnosis, but the full value of this procedure was masked by routine blood smear examinations made on all admissions to the hospital. One striking feature, however, was that over a period of 10 years no endogenous case of *P. vivax* infection was encountered.

Survey findings: Spleens and bloods—Although the spleen rate here (5.6%) was appreciably lower than in the three malarious islands in the Windwards, the general picture was not dissimilar (Tables 5–8). Thus, the parasite rate (adjusted) was higher (7.8%); the predominantly small spleens were usually associated with parasitemia; *P. falciparum* and *P. malariae* occurred in the ratio of 2 to 1, and *P. vivax* was not present.

FIG. 6

The highest spleen rates were found at Freetown (28.7%) and Urlings (22.9%), and rates of 11.4 to 15.4 were recorded at six other localities (Fig. 6), principally in the southern part of the island where the annual rainfall was higher than the general average.

Mansonella ozzardi occurred in 0.6 per cent of the blood smears. With the exception of a Kittitian boy recently domiciled in St. John's, the infection was limited to the south of the island.

Anopheles: Edwards and Box (1940) recorded three species of the *Nyssorhynchus* group in Antigua, viz. *A. albimanus*, *A. tarsimaculatus* and *A. argyritarsis*. Only these species were collected during the present survey, but specimens of *A. tarsimaculatus* were subsequently confirmed as *A. aquasalis* by Shannon (1944).

A. albimanus was the most prevalent (Table 9), being more common in shallow fresh-water ponds and sluggish or stagnant streams. It was nevertheless found in the vicinity of every inhabited locality in the island. It was the only species at Cedar Grove and in the Freetown-Willikies area. *A. aquasalis* was most abundant in brackish coastal swamps and sluggish streams in the east and south, commonly in association with *A. albimanus*. *A. argyritarsis* was collected in the upper parts of the small streams in the south and in rain pools along their banks. Both *A. albimanus* and *A. aquasalis* were occasionally collected in artificial rain-water containers in estate yards.

7. *Nevis: February–March 1947*

Nearly circular in shape, with a total area of 50 square miles, Nevis is separated from St. Kitts by a narrow 2-mile channel. It comprises a central 3,600-foot mountainous volcanic cone from which the land slopes gradually to the sea, scarred by numerous gorges or "ghauts" which are in spate only during the rains, and which are subject to pooling between showers. The seasonal distribution of rainfall was comparable to that for Antigua (Fig. 2). Seepages at Bogg Lands near the main town, and shallow hillside ponds at three settlements—Brown Hill, Pond Hill and Clay Ghaut—were of importance to the malaria problem. Mangrove swamps along the northwest coast were remote from centers of population. A number of the settlements were located above sea level on the slopes of the central mountain, with the houses widely scattered on small peasant holdings.

Malaria prevalence: During the period 1933–42, the average annual malaria morbidity rate in Nevis was 2,029 (Table 3), but as in Antigua the fevers appeared to occur in epidemic form and in localized areas.

Survey findings of spleen and blood examinations are set out in Tables 5–8, and although the spleen (1.9%) and parasite rates (1.9%) were negligible, even here very close positive correlation existed between spleen size and parasitism. *P. malariae* outnumbered *P. falciparum* 3 to 1, but since there were only 22 positive smears this may not necessarily represent the true parasite formula. The highest spleen rates occurred at Camps (17.5%) Jessops (8.9%) and Bath (7.2%); the other 17 localities had rates less than 5 per cent.

Mansonella ozzardi was found in 1.1 per cent of the smears examined—all 4 among residents of Jessops.

Anopheles: Anopheles breeding was present in practically all ground waters existing at the time of survey, but only *A. albimanus* and *A. aquasalis* were collected (Table 9). Whereas the former predominated and was widespread in its distribution, *aquasalis* was found only in 4 areas, the most important of which

was Cotton Ground. Adults of both species were captured off donkey bait in the evening at Camps and Cotton Ground, with densities of 23 *albimanus* and 16 *aquasalis* per man hour.

8. St. Kitts: October, 1943

The island of St. Kitts resembles St. Vincent of the Windwards Group in its dominating extinct 4,314-foot volcano at Mt. Misery and in the light and sandy nature of its soil. There are, however, three small permanent ravines—two at Old Road on the west, and one at Cayon on the east coast; while in the narrow southern end a series of brackish ponds form a striking topographical feature, some of which produce table salt in commercial quantities.

Malaria prevalence: The records of the Medical Department indicated that epidemic malaria occurred at Pond Pasture on the outskirts of the capital Basseterre in 1897, while the presence of anopheles in the island was last recorded by Low in 1902. From the end of the last century, however, St. Kitts has enjoyed a reputation of complete freedom from malaria, and a history of recent domicile in the neighboring island of Nevis was usually obtainable in active cases of malaria treated here. For statistical purposes, therefore, no cases or deaths are recorded for this island in Tables 2–4. In October, 1943, nevertheless, two adult anopheles were captured by a Sanitary Inspector trainee at Conaree, and brief investigations were made by the author shortly after.

Results of reconnaissance: Anopheles—Larvae and pupae of *A. aquasalis* were collected in ponds and seepage areas at Canada and Conaree Estates 29 of 90 observations yielding 56 larvae; and 41 adult *aquasalis* were captured in 7 houses. At Old Road, approximately 9 miles from Conaree and on the opposite side of the island, the two small streams were negative, but recent emergence of one brood was evidenced by the presence of numerous anopheles pupa cases and a single 3rd instar *A. aquasalis* larva in an artificial container at Wingfield Estate. No breeding was found in the stream at Cayon.

Spleen & blood examinations were limited to the two localities positive for *aquasalis*. Although intestinal schistosomiasis is known to occur (Strong, 1945) spleen rates were low and spleens small (Tables 5 and 6). Since one positive smear (*P. malariae*) was found in a child from Nevis and one (*P. falciparum*) in a resident of Conaree who had not travelled away from St. Kitts, further observations were considered desirable.

DISCUSSION

Malaria morbidity and mortality rates presented in Tables 3 and 4 provide a general picture of malaria prevalence in the islands, but based as they are largely on clinical diagnosis, they must be interpreted with caution. It was clear, however, that during the period 1933–1942, St. Lucia, Grenada and Dominica in the Windward Islands had the highest prevalence, and this was confirmed in the survey here reported. Antigua's intermediate position was also confirmed, as was also the relative unimportance of malaria in the other island units. In St. Vincent further observations were indicated to determine more fully the malaria

situation there, while in St. Kitts the local assurance of total freedom was not completely substantiated.

The spleen rates were generally low, but in each island a small number of localities showed evidence of relatively high endemicity (Figs. 3–6). Even in the absence of effective control measures, however, considerable changes in this respect had been observed. Thus, in Grenada, highest rates had shifted from the northeast and north coasts in 1929 (Root & Andrews: 1938) to the middle two-thirds of the east coast in 1945; in Antigua, the spleen rate at Freetown fell from 28.7 in 1943 to 5.4 in 1945. These observations, coupled with the marked fluctuations in reported malaria morbidity and mortality as illustrated in Montserrat, added weight to Earle's (1931) conclusion "that the usual picture is one of localised outbreaks distributed throughout the island (Grenada)".

Nevertheless, reduction of the spleen rate at Vieux Fort in St. Lucia, from 60.0 in 1934 (Earle, 1936), through 15.0 in 1942 (Downs 1942), to 6.0 in 1943, and comparable reductions at Gros Islet and Castries, were the direct results of extensive swamp reclamation by the U.S.A.F. on the one hand, and major control of the lower reaches of the Castries River by the Town Council on the other.

The spleens were predominantly small, nearly half being palpable only on deep inspiration (Table 6). The small number of the largest spleens found all occurred in the three more malarious Windward Islands. There was close positive correlation between splenomegaly and parasitism, and the adjusted parasite rate usually equaled and often exceeded the spleen rate (Table 5). Since this picture is usually associated with acute or subacute and recent enlargement, and since the islands were surveyed at varying seasons, it was concluded that transmission probably occurs throughout the greater part of the year in this area. The value of splenomegaly here as a measure of malaria prevalence was greatly enhanced by the progressive increase of positive smears in direct ratio to spleen size (Fig. 7), and parasitological findings in the PDI spleens served to emphasize that even this group cannot be ignored in assessing malaria prevalence in these islands.

No statistically significant difference in spleen or parasite rate was found by sex or age, or by race, when Negroes and East Indians were observed under comparable conditions. This was probably due to the more severe reactions of the latter group to *P. vivax* infection which was uncommon, and to the preponderance of *P. falciparum*. Indeed, the almost total absence of *P. vivax*, found only in the three larger units of the Windwards, was similar to the findings of Paul and Bellerive (1947) among the predominantly Negro population of Haiti. Furthermore, the lower incidence of this species among Negroes, compared to East Indians in St. Andrews County, Trinidad, B.W.I. (Downs *et al.*, 1943) seems to indicate a degree of Negro immunity to *vivax* in the Caribbean area.

The high *malariae* rate throughout the area was unusual, and was recognised, at least in Antigua and Dominica, as an important factor in the incidence of nephritic states among children. Since vitamin B complex deficiencies in preschool children were frequently associated with edema, blood smear examination was essential in differential diagnosis.

The Malaria vector—Although large-scale dissection of *Anopheles* adults was not carried out, *A. aquasalis* had been previously incriminated as a natural vector by Earle (1936) in Grenada and St. Lucia. Cochrane (1941) considered

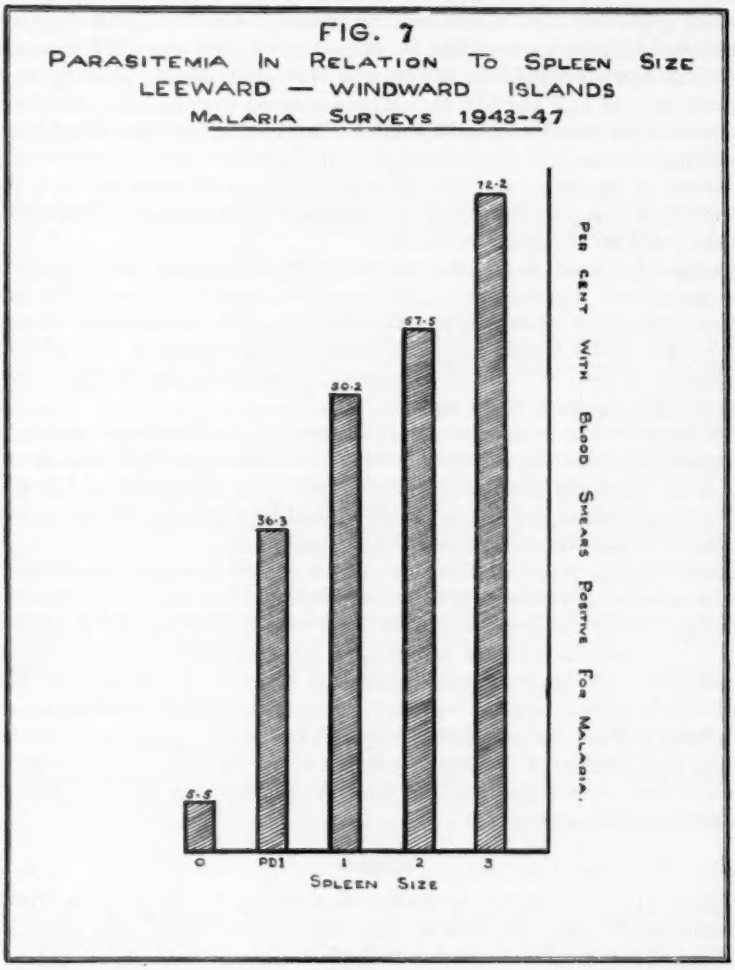

FIG. 7

the possibility of *A. argyritarsis* as an additional vector in Grenada, but both this species and *A. aquasalis* were collected during 1945 in the Tempé Valley (to which Cochrane's 1941 observations referred). *A. aquasalis* is therefore accepted as the only vector in the Windward Islands.

In the Leeward Islands, however, there were epidemiological indications that both *A. aquasalis* and *A. albimanus* contributed to the spread of malaria. Thus, at Cedar Grove on the northern part of the limestone area of Antigua, only *A. albimanus* was breeding during 1943–44 in a series of shallow ponds which were the only possible *Anopheles* breeding foci in that locality, and adult captures in houses and Magoon traps yielded 361 specimens—all *albimanus*. These observations were made towards the end of a severe local epidemic when spleen and parasite rates of 12.7 and 33.6 per cent respectively were recorded. Similarly at Freetown in the southern part of the limestone area of the same island (Fig. 6) *A. albimanus* alone was encountered in the only ground water deposits within a 2-mile radius, and spleen and parasite rates of 28.7 and 28.0 were recorded. These observations were considered highly suggestive of the role of *A. albimanus* in the epidemiology of malaria in Antigua.

On the other hand, at Old Road in the south of this island, *A. aquasalis* was the predominant *Anopheles* species in a mangrove swamp on the outskirts of the village. Although *A. albimanus* breeding occurred a mile inland up the Claremont Valley, of a total 115 adult anopheles taken 108 were *aquasalis*. The spleen and parasite rates were 11.7 and 17.9 respectively, and here the findings were indicative of *A. aquasalis* as the vector.

References to the occurrence of *A. albimanus* in the Windward Islands were not confirmed, either in Dominica or St. Vincent (Senevet 1936). Similar reference to this species in Trinidad by Beattie (1932) was also unconfirmed by subsequent investigations, for Komp (1937) showed that this record was probably erroneous, while Downs *et al.* (1943) failed to collect it.

Control: In the presence of the two vector species *aquasalis* and *albimanus*, control of malaria transmission can be anticipated from residual adulticide applications, for both in Trinidad (1950) and Puerto Rico (Stage and Pratt, 1950) reduction of malaria incidence attributable to these vectors has been obtained by residual DDT. Since malaria distribution is patchy in the Leeward-Windward islands, satisfactory and economic results will probably accrue from residual spraying of the major endemic foci, with concomitant eradication of *Aedes aegypti*. But, because of the zoophilic habits of the local vectors, their eradication will not be achieved without the heavy expenditure usually associated with antilarval control programs.

SUMMARY

Malaria surveys under the auspices of the Colonial Development and Welfare Organisation (British West Indies), were made in the British units of the Leeward and Windward Islands during 1943–47.

Malariometric rates of importance were recorded in Grenada, St. Lucia, Dominica and Antigua where high rates were found in scattered areas. The general picture was one of localized outbreaks distributed periodically throughout each island. Splenometric findings constituted a reliable measure of recent malaria prevalence, and close positive correlation existed between degree of splenic enlargement and parasitemia.

All three of the commoner *Plasmodium* species were present, but *P. falciparum*

predominated; *P. malariae* was common, and *P. vivax* infrequent. *Mansonella ozzardi* was found in Antigua, Nevis and St. Kitts, in addition to those islands previously recorded in the literature.

Four species of *Anopheles* were encountered—*A. aquasalis*, *A. argyritarsis*, *A. albimanus* and *A. pseudopunctipennis*. Previous records of *A. albimanus* in Dominica and St. Vincent were not confirmed. On epidemiological data presented, both *A. aquasalis* and *A. albimanus* appeared to contribute to malaria transmission.

Anticipated results in malaria control from residual DDT are briefly discussed.

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HEPATOMEGALY IN HUMAN AND EXPERIMENTAL AMEBIC COLITIS*

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INTRODUCTION

In the past, parasitologists and clinicians in general have regarded amebic disease of the liver as referring only to the actual invasion of that organ by *E. histolytica*. In recent years, however, various investigators have mentioned the possibility of hepatomegaly in association with colonic amebiasis but without concomitant invasion of the hepatic parenchyma by the parasite. Loeber and D'Antoni (1947) reported their observation of the "frequent finding of a slightly enlarged liver" in a series of 73 children presenting "bizarre and equivocal symptomatology," with cysts of *E. histolytica* in the stools, whose "complaints and abnormal physical findings subsided promptly with appropriate (anti-amebic) therapy." Sodeman and Doerner (1949) found abnormal liver function in 20 per cent of patients with "colonic amebiasis with tender hepatomegaly but without systemic (febrile) response." The number of patients in this group was not stated. In contrast, de Silva (1949) found no clinical evidence of hepatomegaly in 40 children under 2 years of age, with *E. histolytica* in their stools. Sodeman (1950) has described what he called "hepatomegaly in association with colonic amebiasis," reporting that "in many patients with colonic amebiasis we have found some enlargement and tenderness of the liver not associated with any X-ray signs of bulging or impaired movement of the diaphragm and not associated with fever, chills or leukocytosis."

Since the concept of hepatic enlargement due to colonic amebiasis is becoming popular among some clinicians and parasitologists, it has seemed advisable to discover such factual objective information as is available.

MATERIALS AND METHODS

1. *Human.* The hospital records were reviewed of all 25 cases which died with active amebic colitis in the Charity Hospital of Louisiana at New Orleans from 1938 to 1950. Only those from which the following evidence was obtainable were selected for study: age and body weight of subject, weight of liver, and sections of liver and colon for histologic study. Cases which had amebic or other infectious or neoplastic condition of the liver were also eliminated from the series.

For comparison, the weight of the liver in relation to body weight and age was obtained from normal subjects of ages corresponding to those of our cases. These values were secured from the files of Dr. Russel S. Fisher, Chief Medical Examiner, Baltimore, Maryland, based on cases that had died accidentally or by homicide.

* Aided by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, Bethesda, Md.

II. *Experimental.* Eighteen young adult guinea pigs were inoculated intracecally with 100,000 *E. histolytica* trophozoites (Tulane strain 22) cultured in Balamuth's medium. After an incubation period of 7 days the animals were sacrificed and examined grossly and microscopically for the presence of amebic colitis. Only guinea pigs with well developed colonic (cecal) lesions were included in the experimental series. Twelve guinea pigs from the same source, under identical dietary conditions but not infected with *E. histolytica*, served as controls. At the end of the experiment body and liver weights were recorded and sections of liver and colon were prepared for histologic study.

TABLE 1

Weight of liver relative to body weight in human subjects

GROUP A (WITH AMEBIC COLITIS)		AGE (YEARS)	GROUP B (NORMAL)
	per cent		*per cent*
A 38- 833	3.423	74	2.780
A 38-1092	2.684	57	1.833
A 41- 208	2.304	45	2.527
A 46- 911	2.553	72	2.588
A 47- 15	2.634	74	2.780
A 47- 109	3.375	66	1.763
A 47- 719	3.520	45	2.527
Average	2.9276		2.3997

P = 0.05 was obtained using the t-test for statistical significance.*

* Dr. Huldah Bancroft, Professor of Biostatistics, Tulane University, checked the statistical analysis.

RESULTS

I. *Human.* Seven of the 25 cases in which a post-mortem diagnosis of amebic colitis had been made fulfilled the criteria selected for this study. Table 1 shows the weight of the liver as percentage of body weight, both in the amebic cases and in the normal subjects.

II. *Experiments.* The weight of the liver in each animal given as percentage of the body weight is listed in Table 2.

Histologic findings. Sections of colon confirmed the presence of active amebic colitis in the human autopsy material as well as in the infected experimental animals, whereas the control animals had normal intestines. The character of the amebic lesions, however, differed in man and guinea pigs. Those in the human intestine were, in general, less fulminating than in the guinea pig. The latter showed a more acute type of reaction and the lesions were covered with a thick layer of necrotic material such as has been previously described (Carrera and Faust, 1949). Microscopic sections of liver from the human cases of amebic colitis and those from the infected guinea pigs revealed no significant histologic changes, and certainly none attributable to the amebic lesion of the colon. There

were nonspecific findings, such as occasional vacuolization of the cytoplasm of the hepatic cells and mild lymphocytic infiltration of some of the portal spaces, but there was no hepatic necrosis and no other inflammatory reaction. These changes are seen frequently enough in livers from ordinary "normal" autopsy material and in "normal" guinea pigs to justify interpretation of their presence as nonspecific. In the experimental group the gross appearance of the livers varied only in size between the infected and the noninfected animals.

TABLE 2

Weight of liver relative to body weight in guinea pigs

GROUP A (WITH AMEBIC COLITIS)	GROUP B (NORMAL)
per cent	*per cent*
4.4	3.5
3.1	2.9
5.2	3.4
5.7	2.9
4.3	3.5
4.3	3.4
5.0	3.7
3.6	3.8
5.2	4.4
5.9	4.7
7.7	5.2
4.4	4.8
6.7	
5.8	
4.4	
5.1	
5.8	
4.6	
Average.....5.07%	3.85

In this series statistical analysis shows $0.001 < P < 0.01$

SUMMARY

The average weight of the liver relative to body weight in 7 subjects who died with active amebic colitis and without evident concomitant hepatic disease was slightly higher than that of normal subjects of equivalent age. Similarly the average relative weight of the liver in 18 guinea pigs with experimentally induced amebic colitis was higher than in 12 control noninfected animals. The hepatomegaly was more pronounced in the experimental animals than in the human subjects. The character of the amebic colitis was more acute in the guinea pigs. Histologic studies failed to reveal hepatic changes attributable to the colonic amebic infection, nor did it provide an explanation for the hepatomegaly.

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THE EFFECT OF PROTEIN DEFICIENT DIETS ON THE SUSCEPTIBILITY OF GUINEA PIGS TO INFECTION WITH *ENDAMOEBA HISTOLYTICA**

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INTRODUCTION

Ascorbic acid deficiency has been found to play a significant role in the production of experimental amebiasis in the guinea pig (Sadun, Bradin and Faust, 1951). These workers also suggested the possible role of other dietary deficiencies in this disease. In the present study guinea pigs were fed protein-deficient diets and their susceptibility to amebic infection was tested against that of animals fed an adequate diet. Their diets were prepared according to the specifications of Booth, Elvehjem and Hart (1949).

TABLE 1

Composition of diets fed to guinea pigs

	GROUP A	GROUP B	GROUP C
	gm.	gm.	gm.
Purified "vitamin-free" casein	30.0	5.0	0
Zein	0	0	30.0
Sucrose	42.9	67.0	42.9
Salts IV (Hegsted *et al.*, 1941)	4.0	4.0	4.0
Fortified soybean oil (containing 1.2 mg. of carotene, 12 mg. of tocopherol, 8 gammas calciferol, 0.2 mg. of vitamin K)	4.0	4.0	4.0
Gum arabic	15.0	15.0	15.0
Potassium acetate	2.5	2.5	2.5
Magnesium oxide	0.5	0.5	0.5
Sucrose mixture of B vitamins (containing 1 mg. thiamine hydrochloride, 1.4 mg. riboflavin, 1 mg. pyridoxine, 3 mg. calcium pantothenate, 200 mg. inositol, 10 mg. niacin, 10 mg. p-aminobenzoic acid, 0.04 biotin, 0.3 mg. folic acid)	0.8	0.8	0.8
Choline	0.3	0.3	0.3
Total	100.0	100.0	100.0

MATERIALS AND METHODS

Young healthy guinea pigs ranging in weight from 150 to 290 gm. were separated into three groups of approximately the same average weight. The animals were place in individual cages and were fed the special diets† listed in Table 1,

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† Prepared by Nutritional Biochemicals Corporation, Cleveland 5, Ohio

and water *ad libitum* throughout the experiment. A supplement of 20 mg. of ascorbic acid was given by mouth to each animal every other day.

Diet A represented a well-balanced semipurified one for guinea pigs (Booth *et al.*, l.c.) in which the protein was of good quality and adequate in amount. Diet B contained protein of the same quality as Diet A, but the quantity was greatly reduced, while Diet C contained protein equivalent in amount to Diet A, but of known poor quality, notably lacking in lysine and containing only 5.5 per cent of the amount of tryptophane that casein contains (Block and Bolling, 1947). The three diets were otherwise identical and were made isocaloric by adjusting the proportion of sucrose. Bulk and roughage were supplied by gum arabic.

The animals were weighed at the beginning of the experiment and every two days thereafter. Half of the animals in each group were inoculated on the 8th day after being placed on the diet and the rest of the animals on the 11th day. Each animal was inoculated intracecally with 100,000 trophozoites of *E. histolytica* (Tulane strain 22), originally obtained from a human case of amebic brain abscess and maintained in culture in Balamuth's medium. The volume of inoculum was 1 ml.; inoculations were performed following laparotomy under ether anesthesia. Seven days later each animal was sacrificed, and the cecum was examined for the presence of amebic lesions. Wet cover-slip preparations were made from the cecal feces and immediately examined under the microscope for the presence of *E. histolytica*. Portions of the cecal wall were fixed in 4 per cent formaldehyde (USP) solution, from which sections were made for histologic study. Animals that died spontaneously were similarly examined soon after death.

OBSERVATIONS AND RESULTS

Nutrition. After the first 24 hours all animals consumed their diet freely. Fig. 1 shows the average-weight curve for each of the three groups. Animals on diets B and C lost weight rapidly.

Infectivity. Table 2 summarizes the infectivity rate in each group, determined by the finding of *E. histolytica* in the cecal feces and amebic lesions in the histologic preparations of the cecum.

Statistical analysis* of these figures reveals no significant differences between any of the groups. P values for the comparison between groups were as follows:

A vs. C, P = 0.39
B vs. C, P = 0.67
A vs. B, P = 0.70

Pathologic findings. Grossly there was considerable variability in the size and extent of the cecal lesions within each of the three groups and no appreciable difference could be demonstrated from group to group. No microscopic differ-

*Tested as follows: $\frac{X}{\sigma} = \frac{P_1 - P_2}{\sqrt{\frac{P_q}{n_1} + \frac{P_q}{n_2}}}$

ences were noted in the lesions between any two groups. The general histologic pattern of the amebic lesions was similar to that described for guinea pigs consuming a crude commercial diet (Carrera and Faust, 1949).

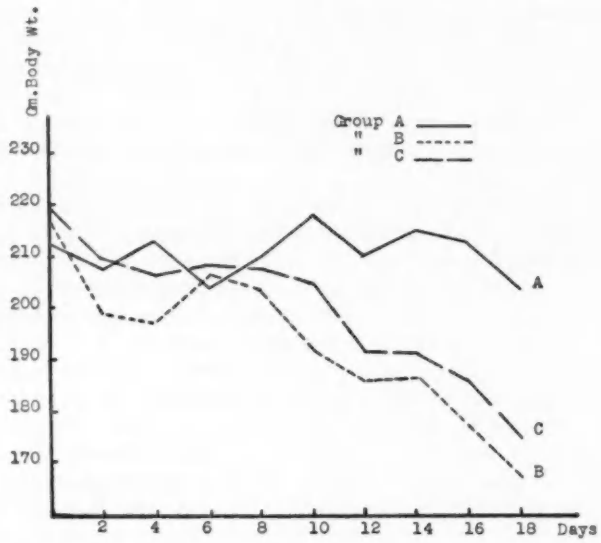

FIG. 1 Average-weight curves of animals in groups A, B, and C

TABLE 2

Number and percentage of guinea pigs infected with E. histolytica

	GROUP A	GROUP B	GROUP C
Number of animals	15	19	20
Infected	11 (73%)	15 (79%)	17 (85%)
Not infected	4 (27%)	4 (21%)	3 (15%)

SUMMARY

Three similar groups of guinea pigs were fed respectively an adequate diet, a diet quantitatively deficient in protein and a diet qualitatively deficient in protein, for a period of 8 to 11 days, after which they were inoculated intracecally with *E. histolytica* trophozoites, were continued on the same diets, and were sacrificed 7 days later. The infectivity rates showed no statistically significant differences, and the gross and microscopic findings were similar in the three groups.

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THE EFFECT OF A BLACKTONGUE-PRODUCING DIET ON EXPERIMENTAL AMEBIASIS IN DOGS

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Although diet has been suspected of playing a role in the severity of human amebiasis (Alexander and Meleney, 1935; Meleney, 1944), little is known of the influence of diet upon the production of lesions and symptoms in infections with *Entamoeba histolytica* (Craig, 1944). So that the findings might have some bearing on the disease in man, it was decided to select for study a deficiency which would meet certain important conditions. It seemed advisable to select one which had been thoroughly studied and well defined both in man and experimental hosts. Moreover, it was necessary that one of the experimental hosts should be suitable for studies on amebiasis. Pellagra and its analogue in dogs, canine blacktongue, have been investigated thoroughly; both diseases are associated with a primary deficiency of nicotinic acid, complicated in many cases by the presence of secondary deficiencies of other B complex vitamins (Margolis *et al.*, 1939; Smith, 1940; Robinson, 1951). In addition, the dog has been shown to be a particularly good host for studies on amebiasis, the intestinal lesions being in general comparable to those in man (Faust, 1932a; Faust and Kagy, 1934a). Although certain substances such as liver, ventriculin, and salmon have been shown to affect the course of *E. histolytica* infections in dogs (Kagy and Faust, 1930; Faust and Kagy, 1934b; Faust *et al.*, 1934), no reports were found dealing with the effects of a specific diet. It was decided therefore that the present study would be designed to test the effect of a blacktongue-producing diet on experimental amebiasis in dogs.

MATERIALS AND METHODS

Mongrel, adult dogs of unknown ages weighing between 5 and 10 kilograms were used in the study. Most of the animals had short hair. These dogs, although in apparent good health, were kept in the laboratory and fed the stock diet for 2 months in an attempt to equalize their nutritional status. During this time they were treated at 3-week intervals with tetrachlorethylene and other anthelmintics until all were negative for helminths except for a few dogs with whipworms. Repeated examinations of material obtained from normal saline enemas failed to reveal amebae or other protozoa.

At the beginning of an experiment, the dogs were matched according to sex and approximate weight and divided into experimental and control groups. The animals were confined individually in large cages and fed a modification of Goldberger's diet (Sebrell *et al.*, 1938). The composition of the diet used is shown in Table 1. The only change in the preparation of this diet was the use of vitamin-free test casein (General Biochemicals, Inc.). All of the dogs were allowed water *ad libitum* and fed daily 2000 grams of the finished diet mixture which totaled 2000 calories (*i.e.*, of the total quantity (600 gm.) of diet components listed in the table,

each dog received 500 gm. daily). Because the deficiency produced by this diet causes, among its various effects, changes in the normal appearance of the intestinal tract, it was necessary to include "diet controls" in addition to the regular controls. The diet controls were handled as the experimental dogs but were not infected. The diet of the regular controls was supplemented with 10 mg. of nicotinic acid given orally twice weekly. This amount had been shown previously to be ample to prevent blacktongue for the period of these experiments (Sebrell *et al.*, *l.c.*). The animals were weighed twice weekly until the experimental and/or diet controls showed a decline in appetite and loss in weight, both early indica-

TABLE 1

*Composition of the blacktongue-producing diet used**

COMPONENTS	QUANTITY	NUTRIENTS		
		Protein	Fat	Carbohydrate
	grams	*grams*	*grams*	*grams*
Corn meal†	400	33.6	18.8	296.0
Cowpeas (*Vagna sinensis*)‡	50	10.7	0.7	30.4
Casein (vitamin-free)§	60	52.0	—	—
Sucrose	32	—	—	32.0
Cottonseed oil	30	—	30.0	—
Cod liver oil	15	—	15.0	—
Sodium chloride	10	—	—	—
Calcium carbonate	3	—	—	—
Total nutrients		96.3	64.5	358.4
Nutrients per 1000 calories		40.1	26.9	149.3

* The corn meal, cowpeas (previously ground coarsely), and salt are stirred into water and cooked in a double boiler of enamelware for about 1½ hours. Then the other components are well stirred in, the total weight being brought to 2400 gm. with water (so that one gm. represents one calorie). The dogs were given 2000 gm. daily of this finished mixture.

† Whole maize meal (white) sifted as for human consumption.

‡ The California black-eyed pea.

§ General Biochemicals, Inc.

tions of a blacktongue attack. Since an attack may develop rapidly, it was necessary at this point to make frequent examinations and daily weighings.

After a variable period of time on this diet (average 10.4 weeks), the experimental dogs showed typical early signs of blacktongue, slight reddening of the buccal membranes with increased salivation, decline of appetite and slight loss in weight. At this stage, an experimental dog and its control were infected. In selecting a method of infection, it was decided to use one by which cultures containing only trophozoites of *E. histolytica* could be given by mouth. Although infections have been produced by giving unprotected trophozoites by mouth (Swartzwelder, 1937), it was felt that more consistent results might be obtained by giving the organisms in capsules which would not open until after passing the stomach. The best and most practical method of those used in preliminary tests was that of gelatin capsules given a thin enteric coating with salol (Goodman and

Gilman, 1941). In several adult dogs given such capsules containing carmine and necropsied one and one-half hours later, the dye was found about one foot above the ileocecal valve and extending from there throughout the large intestine. It was decided on this basis to use such capsules for infecting the dogs of this study. For infecting purposes, 24-hour cultures were used which contained trophozoites of a human strain (H1 or H2) of *E. histolytica*. Each dog received the sediment from 3 cultures (about 6–7 cc., averaging 75 organisms per low power field). This material for each dog was placed in 2–3 large gelatin capsules (No. 13, veterinary), which were then coated very lightly with salol and when dried thoroughly were pushed down the throat of the animal. On the day of infection, animals were fed at 10:00 A.M. and were given the capsules at 4:30 P.M. The dogs were placed overnight in separate, clean cages without litter, to make certain that the capsules were not vomited or passed intact through the intestinal tract. This was verified in all cases.

To prevent exacerbation of the blacktongue syndrome, it was necessary in all cases to administer nicotinic acid to the experimental dogs 2 days postinfection. Minimal amounts were used, since this material is stored for a brief period in the body (Smith *et al.*, 1943). Giving orally 5 mg. per kilogram of body weight for 5 days was usually sufficient to restore normal appetite, etc., and to prevent another attack for 25–30 days (Smith *et al.*, *l.c.*). It was noted, as previously reported, that as the attacks increased in number a longer period of supplementation with nicotinic acid was required to cure the animal, and that the period between attacks decreased (Margolis *et al.*, 1939). In all cases in which the dogs were maintained on the basic diet longer than 11 weeks, all of them were given orally a weekly supplement of one mg. of riboflavin to prevent collapse and death (Sebrell *et al.*, 1938; Margolis *et al.*, *l.c.*). Thiamine as well as riboflavin has been shown to be deficient in this diet, but neither vitamin is significant in the etiology of blacktongue (Margolis *et al.*, *l.c.*). Thiamine supplements were not given in the present study.

To determine the prepatent period, rectal samples were examined directly for the detection of amebae. This material was taken by use of a pyrex glass tube, with a 7 mm. outside diameter and 15 cm. long. One end was tapered smoothly, the other was attached to a syringe bulb of 15 cc. capacity (Faust, 1931). With the tube inserted part way, about 5 cc. of warm physiological saline were forced into the intestine to facilitate the collection of material. These examinations were first made 24 hours after infection and continued daily for one week. By this time, the majority of the dogs had shown trophic amebae on at least 2 occasions. In most cases, from this time until necropsy, the number of examinations per week was reduced to one. However, daily checks were made of some experimental dogs for several days before and after treatment with nicotinic acid to determine the effect on the presence of amebae in the rectal samples (see below).

The dogs were sacrificed during an asymptomatic period or early in an attack to avoid the severe gastro-intestinal involvement, including lesions and dysentery, noted in advanced blacktongue (Goldberger and Wheeler, 1928; Harvey *et al.*, 1938). In each case, the control dog of the pair was sacrificed at the same

time. At necropsy, the liver, the last 6 inches of the ileum and the entire large intestine were removed. The liver was observed grossly, then sliced into thin portions and examined grossly for evidence of abscess formation. In no case was there evidence of such development. The intestine was cut open and examined grossly for amebic lesions (Faust, 1932a; Faust and Kagy, 1934a). Numerous saline smears were made with material scraped from different levels and examined directly for amebae. In addition, a few smears were made from lesions in 3 different dogs to check on the presence of the fuso-spirochetal group of organisms associated with oral lesions in canine blacktongue (Smith *et al.*, 1937). Some of the smears were stained by Gram's method, others with gentian violet. No organisms were observed that resembled these organisms (Smith, 1932). Material was also taken from various levels of the intestine for culture on a modified Ringer's egg media overlaid with Locke's solution, to which sterile rice powder was added at inoculation. These cultures were incubated and examined for *E. histolytica* after 12 hours. If negative for amebae, the cultures were subinoculated daily for 7 days, so that 8 examinations were made before recording the cultures as negative. Although not a routine part of this study, a few slides were prepared of sections from intestinal tissue removed from 2 experimental and 2 control dogs to study the microscopic details of the lesions.

EXPERIMENTAL RESULTS AND DISCUSSION

Experiment 1.—Eighteen dogs were used in the first experiment, 8 each in the experimental and regular control groups and 2 as diet controls. The latter were fed the basic blacktongue-producing diet without supplementation with nicotinic acid, except to cure an attack as described above for the experimentals, and they were not infected with *E. histolytica*. These diet controls were needed to check the effect of the diet *per se* on the appearance of the intestine of the experimental dogs. A small strain of *E. histolytica* (H1) of human origin (diarrheic case) was used in this experiment. The more important findings of the experiment are summarized in Table 2.

The experimental dogs were on the test diet for 9–12 weeks before showing early signs of blacktongue. This period of the initial attack is longer than the 44-day average reported by others (Harvey *et al.*, 1938). The longer period observed here may have resulted from slight differences in the diet and, perhaps more important, from the use of dogs fed an excellent conditioning diet for 2 months before being placed on the deficient diet. It will be noted that one experimental dog, E3, and its control are not listed in the table. These dogs had been on the diet for 11.5 weeks before infection. The experimental dog, after showing amebae in one rectal sample taken 24 hours after infection, died during the night without exhibiting advanced signs of blacktongue. The collapse and sudden death of this animal, and the loss of hair due to a dermatitis failing to heal with mange cure, suggested the cause of death as riboflavin deficiency (Margolis *et al.*, 1939). This prompted the practice mentioned above of giving riboflavin to all dogs after being on the diet for 11 weeks. Postmortem changes were too advanced in E3 to detect living amebae at necropsy the morning following death, but a few scattered

lesions were observed, mostly in the cecum, resembling early amebic involvement (Faust and Kagy, 1934a). The control, C3, sacrificed at this time, having been negative for amebae in one rectal sample, showed no gross lesions and amebae were not detected directly by smears, or indirectly following culturing of material scraped from the mucosal surface at various levels of the large intestine. It was because of the conditions surrounding the death of E3 that these results were withheld from the table.

Of the remaining 7 experimental dogs, 6 first showed amebae in rectal samples from 1 to 4 days after infection for an average prepatent period of 2 days. The other dog, E4, failed to show amebae by this method. Of the 7 controls, however, only 3 (C4, 5 and 7) revealed amebae by ante-mortem examinations and the average prepatent period was 5.3 days. It is important to add that the dogs positive by this method showed amebae only intermittently. In fact, only one animal, E7, was positive consistently. It is also interesting to point out that in 5 cases, when nicotinic acid was given to cure an experimental dog of a blacktongue attack, rectal samples, previously positive for amebae, remained negative for several days.

The animals were sacrificed at varying periods after infection, from 3 to 10 weeks, to obtain a rough estimate of the relation of time and number of amebic lesions observed at necropsy. Two experimental dogs, E1 and 2, and their respective controls were sacrificed after 3 weeks. Of these, only one, E1, was positive for living amebae. However, 3 of these 4 dogs had small amebic lesions; the experimental dogs averaged 33.5 lesions, the controls 3.0. One of the diet controls, sacrificed at this time after 13 weeks on the basic diet, showed minor intestinal changes described by others (see above), but no lesions were observed resembling those caused by *E. histolytica* in the infected dogs and no parasites were detected.

One experimental dog, E4, and its control were sacrificed 6 weeks after infection. While amebae were detected in both dogs in smears and cultures, the experimental animal had 26 lesions, the control only 2. Results somewhat similar to these were obtained for the 8 remaining animals sacrificed 9 or 10 weeks after infection (E5, 6, 7, and 8, respectively, and their controls). All showed living amebae in direct smears and/or cultures, except C8, negative by both procedures. The experimental dogs, with an average of 40.8 gross amebic lesions, had in all cases many more lesions than the controls with an average of 2.8. One of the experimental dogs, E5, had the largest lesion observed in this study. It was about 1 cm. in diameter and was found in the terminal ileum 3 inches above the ileocecal valve, presumably the area of primary colonization. The remaining diet control was sacrificed with E8 and C8. As expected, the changes in the normal appearance of the intestinal tract were more advanced than in the other diet control sacrificed 9 weeks earlier, since the present dog had experienced more attacks of blacktongue. While blood was present in the lumen, no intestinal lesions were observed that could be confused with those produced by *E. histolytica*. The experimental dogs sacrificed 3, 6, 9, and 10 weeks after infection had an average of 33.5 lesions, 26, 41.5 and 40 lesions respectively. It is unlikely, therefore, that the number of lesions was influenced by the period from infection to necropsy.

The results in Table 2 show other details worthy of comment. In considering the controls, 2 dogs (C2 and 8) showed no evidence of infection before or after death. In one case (C2) in which the animal was sacrificed 3 weeks after infection, it is likely that the amebae failed to establish, especially in view of the fact that in another control (C1), sacrificed at the same time after infection, 6 lesions were evident. It is not possible, however, to rule out infection unless sections of the intestine are also negative, as others have found amebae deep in the submucosa beneath healed surface lesions which had otherwise escaped detection (Faust,

TABLE 2

Results of Experiment 1 for dogs on a blacktongue-producing diet (E) and for controls (C) infected with Entamoeba histolytica

DOG NO.	ON DIET BEFORE INFECTION	*E. Histolytica* IN RECTAL SAMPLES TAKEN ANTEMORTEM	PREPATENT PERIOD BASED ON RECTAL SAMPLES	SACRIFICED AFTER INFECTION	NECROPSY FINDINGS		
					E. histolytica in smears	*E. histolytica* in cultures	No. gross lesions observed
	weeks		*days*	*weeks*			
E1	9	+	1	3	+	+	15
C1	9	−	—	3	−	−	6
E2	11	+	3	3	−	−	52
C2	11	−	—	3	−	−	0
E4	10	−	—	6	+	+	26
C4	10	+	5	6	+	+	2
E5	10	+	4	9	+	+	35
C5	10	+	6	9	+	+	3
E6	12	+	1	9	−	+	48
C6	12	−	—	9	−	+	6
E7	12	+	2	10	+	+	39
C7	12	+	5	10	−	+	2
E8	9	+	1	10	−	+	41
C8	9	−	—	10	−	−	0

1932a). The other dog (C8), sacrificed 10 weeks after infection, could have had a low grade infection and undergone spontaneous recovery within this time. The infection of 5 of the 7 controls (71.4 per cent) compares well with the 72.7 per cent infection rate reported for 77 dogs given one infection (Faust, 1932b). In the latter study, 8.4 per cent of the dogs were negative when examined ante-mortem, but showed evidence of infection at necropsy. In the present case, a higher percentage (21.4) of dogs (E4, C1 and 6) showed similar findings.

E. histolytica was demonstrated in smears and/or cultures in all dogs that had been positive by rectal samples taken ante-mortem, except E2. This one failure may have been related to the presence of an intense bacterial infection of unde-

termined etiology involving the lower ileum and entire large intestine which produced a heavy bloody-mucus covering. This material was laden with polymorphonuclear leucocytes. In two dogs (E4 and C6) that had been negative by repeated ante-mortem examinations, *E. histolytica* was demonstrated at necropsy. There is no explanation for the failure to detect amebae before death in these cases, unless their numbers were too small to be demonstrated by the methods used or because of the location of the lesions. Of the 12 infected dogs, only one, C1, failed to reveal *E. histolytica* before and after death. The results in Table 2 also show for the 14 dogs a much greater percentage of positive findings for *E. histolytica* in cultures (71.4) than in direct smears (42.9).

Experiment 2.—Since there is evidence that the pathogenic index of *E. histolytica* varies with strains (Meleney and Frye, 1933), it was decided to repeat Experiment 1 using another human strain from a diarrheic case (H2). This ameba was within the usual size range. In other respects, the design of this experiment was

TABLE 3

Summary of Experiments 1 and 2. The experimental and control dogs were on their respective diets for an average of 10.4 weeks before being infected with Entamoeba histolytica

	TOTAL NO. DOGS	PER CENT POSITIVE BY RECTAL SAMPLES TAKEN ANTEMORTEM	AVERAGE PREPATENT PERIOD BASED ON RECTAL SAMPLES	AVERAGE TIME SACRIFICED AFTER INFECTION	NECROPSY FINDINGS			
					Per cent positive		Gross lesions	
					E. histolytica in smears	*E. histolytica* in cultures	Per cent with lesions	Average number
			days	*weeks*				
Experimental dogs	17	88.2	2.1	9.2	58.8	82.4	100.0	31.5
Control dogs	17	41.1	4.9	9.2	35.3	58.8	70.6	3.8

the same as that described above for Experiment 1. Ten dogs each were used in the experimental and regular control groups, and 2 dogs were employed as diet controls. None of the animals died during the experiment, which may have been due to the weekly supplementation of riboflavin after 11 weeks on the diets. The results of this experiment resembled so closely those of Experiment 1 that it seemed unnecessary to list them separately. Instead, the important results of both experiments were combined for inclusion in Table 3.

In the 2 experiments, 15 of the 17 experimental dogs (88.2 per cent), on the basic diet for an average of 10.4 weeks before infection, showed trophic amebae ante-mortem, while less than half that number of controls (41.1 per cent) were positive by this procedure. The percentage for the controls is considerably less than that (87.0) obtained by another worker (Faust, 1932b). However, the 2 studies cannot be compared directly because of differences in the strain of ameba used, the method of infection, etc. Perhaps of greatest significance was the use of dogs harboring various parasitic organisms, as compared with the present study in which most of the dogs were rendered parasite-free prior to use. This may be a lead worth future consideration in view of the evidence that concurrent infections often lower resistance.

The prepatent period was much shorter in the experimentals than in the controls (2.1 and 4.9 days respectively). These periods are quite similar to the average period (3.6 days) reported for human strains in another study, in which a different method of infection was employed (Faust, 1932a). Although no record was kept in the present study, the density of amebae in the rectal samples appeared to be much greater in the case of the experimental dogs.

At necropsy, performed on the average 9.2 weeks after infection, *E. histolytica*, in the trophozoite stage, was demonstrated in smears in 58.8 per cent of the experimental dogs, compared with 35.3 per cent of the controls. The value of cultures in increasing the number of positives revealed by direct smears is shown clearly, since there was a 40.1 per cent increase for the experimental animals (from 58.8 to 82.4 per cent), and a 66.6 per cent increase for the controls (from 35.3 to 58.8 per cent).

Every experimental dog had amebic lesions, the average being 31.5, whereas only 70.6 per cent of the controls had such lesions, and, more important, they averaged only 3.8 lesions each. Although the dogs were kept on their diets until necropsy, there was no evidence of a relationship between the number of lesions and the time of necropsy following infection. The infection rate for the controls (70.6 per cent) resulting from one infection compares very closely with 72.7 per cent reported for a series of 77 dogs (Faust, 1932b). In all of the dogs of the present study, the greatest number of lesions was observed in the cecum, with the rectum and colon next in order. Study of a few slides with sections prepared from intestinal tissue of 2 experimental and 2 control dogs revealed microscopic details of the lesions similar to those reported by others in extensive studies (Faust and Kagy, 1934a).

Student's "t" test was the statistical instrument used to analyze the results of both experiments. Comparisons were made of the observed differences between the experimental and control dogs as to positive rectal samples taken antemortem, the prepatent periods, and the various necropsy results. In all cases, the observed differences shown in Tables 2 and 3 were found to be highly significant, *i.e.*, significant at least at the one per cent level. (All data are on file, Department of Parasitology, University of North Carolina.)

The above results show that the control dogs of this study responded to infection with *E. histolytica* in a manner similar to that described by earlier workers for dogs on an adequate diet. Likewise, the effects of the blacktongue-producing diet were comparable to those produced by similar diets used in many other studies. Thus, the results for the experimental dogs permit the conclusion that this deficient diet lowered markedly their natural resistance to *E. histolytica*. It will be recalled that the experimental animals and controls were fed the same diet, the only difference in treatment being the administration to the controls of adequate amounts of nicotinic acid. Neither group received supplements of vitamin B_1 and both were given the same quantity of riboflavin supplements. Therefore, the fact that this diet is deficient in thiamine and riboflavin (Margolis *et al.* 1939; Smith, 1940) would not appear to interfere with the conclusion that the lowered resistance of the experimental dogs was related to a deficiency of nico-

tinic acid alone. In this respect, it is of great interest that raw liver or liver extract, used successfully in curing blacktongue (Harvey *et al.*, 1938), had an ameliorating effect on the course of *E. histolytica* infections in dogs on an adequate diet (Kagy and Faust, 1930; Faust and Kagy, 1934b). Moreover, liver extract given parenterally was only partially successful, even in huge dosage, in the cure of blacktongue (Harvey *et al.*, *l.c.*), and when given in this way it did not control amebic infection (Faust and Swartzwelder, 1936). This would seem to increase the suspicion gained from the present study that nicotinic acid in dogs is in some way involved in the mechanism of resistance to this parasite. In this light, it is worth recalling an observation of the present study. Following treatment of a blacktongue attack with nicotinic acid alone, *E. histolytica*, previously present, could not be found in rectal samples for several days.

Care must be exercised in relating findings in lower animals to man. Thus, it is not to be implied from the above results that pellagrins, or those on diets deficient in the pellagra-preventing factor, are more susceptible to infection with *E. histolytica*, or that they are likely to develop more acute infections. However, it is tempting to point out that the observations of one authority on pellagra have allowed this tentative hypothesis (Smith, 1952). This relationship, therefore, would appear to be worthy of further consideration.

SUMMARY

Two experiments were performed in which a total of 17 dogs were fed a blacktongue-producing diet and 17 controls were fed the same diet but given adequate supplements of nicotinic acid twice weekly. The dogs, after being on their respective diets for an average of 10.4 weeks, were infected orally with trophozoites of *Entamoeba histolytica*, given in salol-coated gelatin capsules.

A much greater percentage of the experimental dogs than of the controls was positive for amebae in rectal samples taken ante-mortem (88.2 and 41.1 respectively), and the average prepatent period was considerably shorter in the experimental animals (2.1 and 4.9 days, respectively). At necropsy, performed 3–10 weeks after infection, *E. histolytica* was demonstrated in 82.4 per cent of the experimental dogs and in 58.8 per cent of the controls. All of the experimental dogs had amebic lesions, with an average number of 31.5 lesions, whereas only 70.6 per cent of the controls had lesions, the average being only 3.8.

Since the experimental dogs and controls were handled similarly, except for supplements of nicotinic acid given to the controls, it would appear that in this host nicotinic acid is involved in the mechanism of the resistance to *E. histolytica*. Other experimental studies in dogs are cited which seem to have a bearing on this conclusion. In addition, observations of one authority on pellagra suggest that these results might have a similar relationship in man.

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TREATMENT OF A CASE OF BALANTIDIAL DYSENTERY WITH TERRAMYCIN

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Infections with *Balantidium coli* have been found to respond favorably to carbarsone (Young and Burrows, 1943) and diiodohydroxyquinoline (Diodoquin) therapy (Shookhoff, 1951). A few reports have recently appeared which describe the use of antibiotics for balantidiasis. Successful treatment was obtained with bacitracin (Castellanos, del Prado, Montero, and Montero, 1951), and aureomycin (Santos, 1951). Chilean investigators have reported that both aureomycin and terramycin were balantidicidal *in vitro* (Agosín, Christen, and Rubio, 1950; Neghme, Agosín, Christen, and Rubio, 1952), and treated four cases with aureomycin and one with terramycin with rapid clinical improvement in all (Neghme, Miranda, Agosín, and Sanz, 1951). The following is a report of a case of balantidial dysentery which we observed, and which showed a similarly favorable response to terramycin.

The patient was a 35 year old Korean prisoner of war. As a civilian, he had been a farmer and had had contact with pigs. He was admitted to the 64th Field Hospital on October 23, 1951 with a history of two weeks of abdominal pain and tenesmus, with mucus and blood present in the stool.

Two weeks previous to his admission to the hospital he had a sudden onset of abdominal cramps and tenesmus; his stools became soft and contained small amounts of mucus and blood. Physical examination was essentially negative except for slight tenderness in the right lower quadrant. Sigmoidoscopic examination revealed mild hyperemia of the rectal mucosa, with a mucus exudate. Ulcerations were not seen, nor was pus visible macroscopically or microscopically.

Stool examination revealed trophozoites of *Balantidium coli*. At this time the stools were semisolid in consistency and contained small amounts of blood and mucus; they averaged 3 to 4 a day with as many as 12 stools on a single day. Rectal swabs cultured at this time for bacterial enteric pathogens were negative, but daily stool examinations were consistently positive for *B. coli*. On several occasions, all the trophozoites seen were filled with great numbers of red blood cells in various stages of digestion. Cysts of *B. coli* were never encountered, nor were other species of intestinal protozoa.

On the fifth hospital day the patient had a chill and temperature elevation to 104°F., and *Plasmodium vivax* was demonstrated in a blood film. Atabrine treatment was begun, 3 grains t.i.d. on the first day, and 1½ grains t.i.d. for 5 days thereafter. During this period daily stool examinations were all positive for *B. coli*. On November 5, the day after he had received his last dose of atabrine, the patient was started on terramycin.[4] Two grams were administered the first day

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[4] We are indebted to the Charles Pfizer Co. for the supply of terramycin hydrochloride.

in a single dose, followed by one-half gram t.i.d. for 10 days thereafter to make a total of 17 grams. *B. coli* was found on stool examination November 6. However, in no instance were trophozoites or cysts recovered in 17 additional stools examined between November 7 and November 29, 8 by saline smear and 9 by zinc sulfate centrifugal flotation.

Immediately after terramycin therapy was begun the stools became fewer in number, fluctuating between 1 and 3 a day; they still on occasion contained mucus but no blood. The patient continued to complain somewhat of low abdominal cramps and slight tenesmus for two weeks following the initiation of therapy. By the fourteenth day, however, the stools became formed, with only one passage a day, and the patient became asymptomatic.

Sigmoidoscopic examination performed one day after conclusion of terramycin therapy revealed some mild hyperemia and edema of the rectal mucosa with a few scattered raised patches of ecchymosis 5 to 10 mm. in diameter. No *Balantidium* or other protozoa could be demonstrated in aspirates from these areas. Bacterial cultures made from swabs taken through the sigmoidoscope were negative for enteric pathogens. Eight days later sigmoidoscopic examination revealed no abnormalities. The patient had to be discharged from the hospital 14 days after therapy was concluded and no further examinations were possible, but at this time he was negative.

Although atabrine was given prior to treatment with terramycin, there is no reason to believe that it influenced the course of the balantidial infection inasmuch as many trophozoites continued to be found in the feces during the entire period that it was administered. In addition, Shookhoff (1951) has treated one case of balantidiasis with atabrine with no success. This leads us to believe that the rapid disappearance of the *Balantidium* and the signs of clinical improvement which followed terramycin therapy were due to the action of this drug alone.

SUMMARY

A case of balantidial dysentery in a Korean responded favorably to terramycin given in divided doses over a 10 day period to total 17 grams. Trophozoites of *Balantidium coli* disappeared after the second day of treatment.

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THE TROPICAL RAT MITE (*LIPONYSSUS BACOTI*) AS AN EXPERIMENTAL VECTOR OF COXSACKIE VIRUS[1]

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It has been repeatedly shown that strains of Coxsackie virus may be isolated from stools and throat washings of the human population (Dalldorf *et al.* 1949; Huebner *et al.* 1950; Sulkin *et al.* 1950; Melnick *et al.* 1949). The fact that this agent has also been demonstrated in sewage (Melnick *et al.* 1949; Clark *et al.* 1951) and in flies (Melnick *et al.* 1949; Ward 1952) has suggested that flies might play a part in the dissemination of the virus. Recently Fischer and Syverton (1951) showed that the cockroach can serve as an experimental vector, Coxsackie virus being isolated from the excreta of roaches for periods of as long as 15 days following a single virus meal. The demonstration by Morris (1951) of Coxsackie virus neutralizing antibodies in sera from 15 of 57 wild rabbits, 1 of 2 chipmunks, and 1 of 13 marmots suggests that infection with these viruses is not limited to the human population. It would be of interest to know whether these presumed Coxsackie infections were spread through the rodent population by animal-to-animal contact or through the intervention of some ectoparasite.

In this laboratory, work with the members of the Coxsackie group has been constantly in progress. During a period of approximately two months infestation of animals and laboratory personnel with the tropical rat mite, *Liponyssus bacoti*, occurred. All of the laboratory workers received multiple mite bites and one individual showed considerable sensitivity as evidenced by the development of urticarial wheals and papules. Although the infestation was eliminated as soon as possible it nevertheless seemed important to establish whether or not these mites could transmit Coxsackie virus. Not only was it of interest to evaluate the ability of an ectoparasite to transmit Coxsackie virus but also the presence of an agent capable of transmitting the virus could have resulted in mixing of the strains in the laboratory or the development of false positive tests. The possibility of infection of laboratory workers through the mite bite also had to be taken into consideration. Perhaps ectoparasites were involved in one or more of the cases of laboratory infection with Coxsackie virus which have been reported (Findlay *et al.* 1950; Shaw *et al.* 1950).

This report deals with experiments designed to evaluate the possible role of *Liponyssus bacoti* in the transmission of Coxsackie virus.

MATERIALS AND METHODS

Mites identified as *Liponyssus bacoti* were first collected from the woodwork and laboratory benches when they emerged from the cracks in search of

[1] Aided by a grant from the Caruth Foundation.

food. Later an infested nest of wild mice was found in a discarded box and the mice and their bedding were maintained in an enamel pot surrounded by a water moat as a constant source of mites. In one instance mites were collected from cages of experimental mice in the laboratory. When the mites were to be used for transmission experiments they were placed in lots of 15 to 20 in small glass tubes one end of which was covered with fine-meshed bolting silk attached by Duco cement. A tight-fitting rubber stopper was inserted in the other end. For feeding of the mites mice were inserted in the tubes. In later experiments the heads of the mice were excluded from the tubes by placing them through a small opening in a piece of rubber dam which was stretched over the end of the

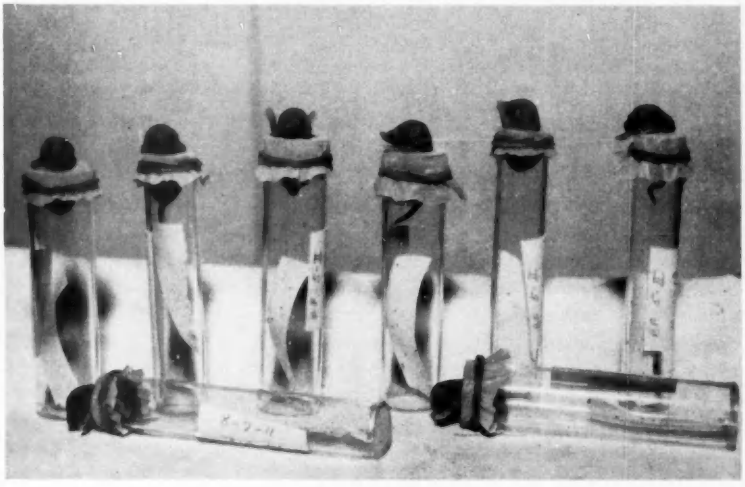


FIG. 1. Method used for feeding mites on suckling mice

tube (Figure 1). The tubes of mites were kept at room temperature in covered jars containing a small amount of water.

The Coxsackie virus used was the Dallas M. B. strain (Sulkin *et al.* 1950), a group A strain serologically similar to the Texas-1 strain. This strain is quite stable at room temperature and has been found to maintain its virulence after suspension in 0.5% phenol for 28 days at room temperature. Suckling mice can regularly be infected percutaneously by lightly scarifying the skin and swabbing the area with a virus suspension.

In order to determine the ability of *Liponyssus bacoti* to transmit Coxsackie virus by bite, adult mites were allowed to feed on mice which were infected with the Dallas M. B. strain. (Blood from these mice gave a virus titer of 10^{-7} when titrated in suckling mice.) The engorged mites were then collected and allowed to feed on normal mice at later periods. When it was desirable to test for the presence of virus in the mites they were ground and suspended in sterile saline

and the suspension was treated with streptomycin and penicillin to render it free of bacteria. Mice were then inoculated subcutaneously with such suspensions. All of the mice used for injection or transmission experiments were 2-day-old suckling white Swiss mice (CF-1). Animals which had received mite bites or were inoculated with mite suspensions were observed daily until the development of symptoms of Coxsackie infection or for a period of 14 days. When no symptoms were observed during the first week, at least one mouse was sacrificed from each group for a "blind" passage to test for the presence of latent virus.

RESULTS

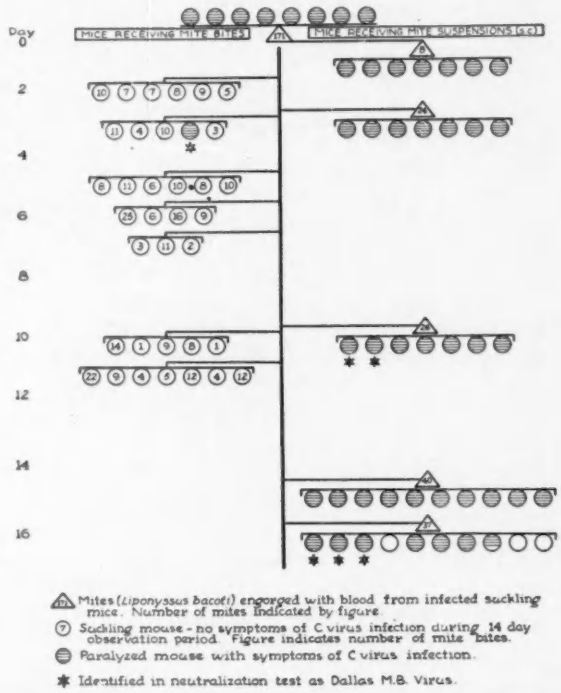
Attempts to isolate Coxsackie virus from presumably normal rat mites—A total of 188 mites were used for the injection of mice to test for the presence of Coxsackie virus. They represented mites trapped at random in the laboratory, those collected from adult mice which were being immunized against strains of Coxsackie virus, and those obtained from a wild mouse nest. In testing the mites obtained from the mouse colony the mite suspension was administered orally and intranasally to a litter of mice in addition to the subcutaneous injection of other litters. In no instance did mice inoculated with these mites develop any illness during a 2 week period of observation. "Blind" passage of muscle tissue from various groups of mice also yielded no evidence of Coxsackie virus infection. The presence of antibody-containing mouse blood in mites collected from animals being immunized against certain strains of C virus was not ascertained.

Survival of Coxsackie virus in mites—Virus was demonstrated in adult mites by inoculation of mice with mite suspensions made 1, 3, 10, 15, and 16 days following their infective blood meal (Chart 1). Tests for the survival of virus in the mites at periods later than 16 days were not carried out. The agents recovered from the mites sacrificed on the 10th and 16th days were identified in neutralization tests with specific antisera as the Dallas M. B. strain of Coxsackie virus.

Attempts to transmit Coxsackie virus by mite bite—The results obtained when Coxsackie-fed mites were allowed to feed on normal mice are shown in Chart 1. Of 36 mice which received multiple bites by infected mites from 2 to 16 days following the infection of the mites only one mouse which was bitten on the third day developed Coxsackie infection. This animal showed typical symptoms on the sixth day after exposure and the agent recovered was identified by a neutralization test as the Dallas M. B. strain. The remaining four mice bitten on the third day failed to develop illness when observed for 10, 14, 14, and 14 days, respectively, and blind passage of muscle tissue failed to show evidence of a latent infection. It is possible that the one positive mouse developed infection from ingesting an infected mite rather than from being bitten since at the time of this feeding the heads of the mice were not excluded from the mite tubes. It has been shown that suckling mice may be infected by oral administration of Coxsackie virus (Kaplan *et al.* 1951). A subsequent attempt to infect seven mice by administering a virus-containing mite suspension orally and intra-nasally was unsuccessful, however, as was the feeding of whole infected

mites to five other mice. All of the mice bitten on the 2nd, 5th, 6th, 7th, 10th, and 11th days (a total of 31 mice) failed to show evidence of Coxsackie infection when observed for 14 days or when tested by "blind" passage.

Test of progeny of infected mites for transovarian passage of virus—Two groups of eggs were collected from the virus-fed mites: those laid during the first 48 hours after the infective blood meal and those laid four days later. The progeny were reared through the first nymphal stage and were then sacrificed and used


Day
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MICE RECEIVING MITE BITES
MICE RECEIVING MITE SUSPENSIONS (I.C.)
Mites (Liponyssus bacoti) engorged with blood from infected suckling mice. Number of mites indicated by figure.
Suckling mouse - no symptoms of C virus infection during 14 day observation period. Figure indicates number of mite bites.
Paralyzed mouse with symptoms of C virus infection.
Identified in neutralization test as Dallas M.B. Virus.

CHART 1. Exposure of suckling mice to the bites of tropical rat mites (*Liponyssus bacoti*) infected with Coxsackie virus (Dallas—M. B. strain).

for the injection of mice (110 nymphs were obtained from the first group; 37, from the second group). In neither instance was evidence obtained that the progeny had harbored Coxsackie virus.

SUMMARY AND CONCLUSIONS

The tropical rat mite, *Liponyssus bacoti*, was shown to harbor the Dallas M. B. strain of Coxsackie virus for as long as 16 days following an infective blood meal. Of 36 mice exposed to the bites of infected mites during this period only one mouse, which was bitten on the third day, developed Coxsackie infec-

tion. The possibility that this animal was infected by ingesting a mite rather than by being bitten by one cannot be excluded. The fact that other mice bitten on the same or closely adjacent days did not develop infection tends to suggest that such might have been the case. Limited tests with progeny from the infected mites did not indicate that transovarian passage of the virus occurred. Thus it would seem that the mite, *Liponyssus bacoti*, can play but a small role, if any, in the transmission of the strain of Coxsackie virus tested.

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EFFECT OF "B" VIRUS (STRAIN NO. 1) IN THE SYRIAN HAMSTER

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The "B" virus (strain no. 1) was isolated from the spleen and central nervous system of patient B and maintained since the original isolation in 1932 by serial passage in rabbits. The virus is extremely pathogenic for rabbits by most routes of inoculation. Following intracutaneous injection, a hemorrhagic necrotic area develops around the site of inoculation. About one to two days before paralysis occurs, most rabbits exhibit a tendency to bite the local lesion (Sabin and Wright, 1934). The present study was undertaken to compare the susceptibility of Syrian hamsters exposed by various routes to the rabbit-adapted strain and mouse-adapted strain (Reagan *et al.*, 1952) of the virus.

METHODS

The virus employed in this study was the "B" virus (strain no. 1) furnished by the American Type Culture Collection in Washington, D. C. This virus had originally been obtained by the above institution from Dr. Albert B. Sabin of the Childrens Hospital in Cincinnati. Before receipt at this laboratory the virus had been passed 67 times in rabbits. Lyophilized rabbit spinal cord of the sixty-seventh passage was received at this virus laboratory and was diluted to a 10 per cent suspension with physiological saline.

EXPERIMENTAL

Three albino rabbits (weight 2 lbs.) were inoculated intradermally with 0.1 cc. of this 10 per cent suspension. The rabbits showed paralysis, with a hemorrhagic necrotic area around the site of infection and nervous symptoms, in 7 days. When symptoms appeared the rabbits were sacrificed and the spinal cords removed aseptically. Six Swiss albino mice were inoculated intracerebrally with 0.03 cc. of a 10 per cent suspension of the 11th intracerebral mouse passage of "B" virus. The mice showed nervous symptoms on the fifth day. When these symptoms of central nervous system involvement appeared, the mice were sacrificed and the brains were removed aseptically. The pools of the infected rabbit cords and infected mouse brains were stored in the $-40°C$. CO_2 deep freeze storage vault. Two weeks later, the 2 pools were removed from the deep freeze, ground with alundum, and diluted to 20 per cent suspensions with physiological saline. The 2 suspensions were then subjected to 5 minutes centrifugation in an angle centrifuge at 2,000 r.p.m. The supernatants were used as the inoculum for all methods of exposure in hamsters. Material from each supernatant was confirmed to be "B" virus by neutralization test in rabbits intradermally. After 6 days, the rabbits inoculated with virus and normal

serum showed a hemorrhagic necrotic area around the site of inoculation, intense scratching, and nervous symptoms; while the rabbits inoculated with virus and immune serum appeared normal. This confirmed the virus in both pools to be "B" virus (strain no. 1).

Thirty-six healthy Syrian hamsters (weight 20 g.) were divided into 9 groups of 4 animals each. The 4 hamsters of each group were administered the rabbit cord virus by one of the following methods: intracerebral, intraperitoneal, intradermal, intramuscular, intralingual, intratesticular, and intracardiac injection and rectal and intranasal instillation. The inoculum consisted of 0.1 cc. of the above described suspension for all routes of exposure except for the intracerebral, intranasal, and intralingual groups where the inoculum was 0.03 cc. A second series of thirty-six healthy Syrian hamsters (weight 20 g.) were

TABLE 1

Response of Syrian hamsters to "B" virus, strain no. 1

ROUTE OF INOCULATION	NO. ANIMALS EXPOSED		NO. SHOWING SYMPTOMS		*MIN. TO MAX. INCUBATION PERIOD (IN DAYS)	
	Rabbit-adapted "B" virus (cord)	Mouse-adapted "B" virus (brain)	Rabbit-adapted virus	Mouse-adapted virus	Rabbit-adapted virus	Mouse-adapted virus
Intraperitoneal	4	4	4	4	4 days	3 days
Rectal	4	4	4	4	4 days	3 days
Intradermal	4	4	4	4	4 days	3 days
Intracardiac	4	4	4	4	3 days	3 days
Intramuscular	4	4	4	4	3–4 days	3 ays
Intralingual	4	4	4	4	3 days	3 days
Intratesticular	4	4	4	4	4 days	4 days
Intracerebral	4	4	4	4	2 days	2 days
Intranasal	4	4	4	4	3–4 days	4–5 days

* Incubation period = time between exposure to virus and onset of symptoms.

divided into 9 groups of 4 animals each and were treated in like manner using the mouse-adapted "B" virus. After inoculation all groups were observed twice daily for nervous symptoms characteristically found in mammals infected experimentally with "B" virus.

After nervous symptoms and paralysis appeared, the animals were sacrificed. The brains were removed from each group separately, pooled, and frozen in a −40°C. CO_2 deep-freeze storage vault. After all animals had succumbed to the disease, the pools were removed from the deep freeze, thawed, and ground to 10 per cent suspensions with physiological saline. The virus contained in these suspensions was confirmed to be "B" virus by neutralization tests using normal and hyperimmune guinea pig sera. Hamsters were used as the test host and were injected intradermally.

RESULTS AND DISCUSSION

Table 1 shows the response of Syrian hamsters to the rabbit-adapted and mouse-adapted "B" virus (strain no. 1) inoculated by different routes. The

incubation period in hamsters from both the rabbit-adapted virus and the mouse-adapted virus was from 2 to 4 days, the pericd varying slightly with the route of exposure. All of the hamsters injected, except for the intracerebral series from each pool, showed paralysis of the hind legs and intense scratching around the inoculation area. In the intracerebral series the hamsters became prostrate before paralysis could be noted. The virus in each pool of hamster brains was confirmed to be "B" virus by the neutralization tests.

SUMMARY

A strain of rabbit-adapted "B" virus (Sabin and Wright, 1934) and of mouse-adapted "B" virus (Reagan *et al.*, 1952) have been successfully transmitted to the Syrian hamster by the following routes of exposure: intracerebral, intraperitoneal, intradermal, intracardiac, intralingual, intratesticular, intramuscular, intranasal, and rectal. Hamsters responded to the mouse-adapted "B" virus equally as well as to the rabbit adapted "B" virus.

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SURVIVAL OF INGESTED *SALMONELLA* IN THE COCKROACH *PERIPLANETA AMERICANA*

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While there is as yet no unequivocal proof that cockroaches are biological vectors in the transmission of *Salmonella* infections to man, these insects have been implicated on the basis of circumstantial evidence obtained during the investigation of epidemics (Scott, 1915; Antonelli, 1930; Mackerras and Mackerras, 1949) as well as by the finding of naturally infected specimens in the immediate vicinity of known cases of salmonellosis or in communities where human enteric infections were common (Scott, 1915; Mackerras and Mackerras, 1948; Bitter and Williams, 1949). It has also been shown that roaches can be infected in the laboratory by feeding them *Salmonella* and that the insects will subsequently shed the micro-organisms in their feces for a few or many days (Longfellow, 1913; Tejera, 1926; Spinelli and Reitano, 1932; Mackerras and Pope, 1948; Wedberg *et al.*, 1949, 1951; Olson and Rueger, 1950) although some of the attempts to induce such experimental infections, notably with *S. typhosa*, have been largely or wholly unsuccessful (Macfie, 1913; McBurney and Davis, 1930; Janssen and Wedberg, 1952). Regrettably, certain of the published reports fail to state the species of cockroach examined or the number of viable *Salmonella* administered, while in other studies it appears that many millions of organisms from pure cultures were given. There is a serious lack of quantitative data concerning the ease with which various species of roach can be infected by moderate numbers of *Salmonella*, data which might be very helpful in evaluating the possible role of cockroaches in human salmonellosis. Thus far efforts to determine the order of magnitude of the minimal infective dose have been made by few if any workers other than Wedberg *et al.* (1949), who found that it was necessary to feed at least 5000 *S. typhimurium* in order to obtain infection of *Blaberus cranifer*.

The experiments described below were performed, largely during the Spring of 1950, in order to provide additional information and were based on the assumption that infected human excreta might be one of the sources of *Salmonella* readily available to roaches. It was decided to employ *Periplaneta americana* in the study because of the known association of this species with the interior of human habitations as well as with privies, sewers and other outdoor sources of dejecta. The size of the adult insects also permitted convenient handling. The feeding of measured doses of the micro-organisms suspended in feces seemed to us preferable to the procedures used by others which involved administration of patients' stools containing unknown numbers of *Salmonella* or of pure cultures suspended in a nutrient fluid. Attempts were therefore made to determine a) the average quantity of human feces consumed by individual *P. ameri-*

cana at a single feeding; b) the duration of survival of *Salmonella* in the enteric canal of roaches following ingestion of feces containing varying numbers of these bacteria. Experiments were also performed to learn how long *Salmonella* might persist in human feces since this would give some idea as to the extent of opportunity which might be afforded roaches to acquire infection from such sources.

The findings reported below should provide a baseline for further work which might attempt to measure the span of time over which roaches, given orally small rather than large multiples of the minimum infective dose, may continue to harbor and excrete *Salmonella* in their feces, particularly when the animals are allowed to feed on various substrates at intervals following inoculation. The protracted survival of *Salmonella* in cockroach feces has already been convincingly demonstrated (Olson and Rueger, 1950).

MATERIALS AND METHODS

Collection, handling and bacteriologic study of cockroaches

Adult *P. americana* were trapped in the immediate environment of the institution by means of clean jars coated within by a film of glycerin and baited with molasses. They were transferred daily for a week to clean sterile flasks in which they were deprived of food but had access to water. The roach feces deposited were collected by rinsing the flasks with sterile 0.85 per cent sodium chloride solution and were cultured for *Salmonella* (*vide infra*), with invariably negative results. The roaches were then offered feces artificially inoculated with *Salmonella*, which they ate abundantly in all cases. At the end of an hour, when feeding had almost always ceased, they were removed to fresh sterile containers and kept at room temperature (15°–25°C.) for varying periods of time until they were immobilized by chilling preparatory to dissection.

After the legs, wings and antennae had been removed each insect was dipped in 70 per cent alcohol, allowed to drain and when practically dry was placed ventral side up. Bilateral incisions were made from the neck to a point just below the anus, so that the entire ventral body wall could be lifted forward. With fresh sterile instruments the gastro-intestinal tract was lifted out, severed at oesophagus and rectum, then placed in a sterile mortar and thoroughly ground with 1 to 2 ml. of saline. The resulting suspension was cultured (at 37°C.) by a procedure which included: preliminary enrichment overnight in tetrathionate broth containing 0.001 per cent brilliant green; plating of a sample of this broth culture on Difco S S agar; isolation of pure cultures from colonies on the S S plate which were suspected of being *Salmonella;* identification of cultures giving typical reactions on Difco triple sugar iron agar (TSIA) and study of their behavior in appropriate biochemical and serologic tests.

To obtain an estimate of the quantity of feces ingested at a single feeding, roaches which had been starved for a week were divided into two groups, the members of each showing the same range of body size and weight. The individuals of one group, unfed, were dissected and the weight of the alimentary tract determined to the nearest milligram. The second group was allowed to feed on feces for an hour, then the weight of the alimentary tract of each indi-

vidual was similarly measured. An approximation of the quantity of feces ingested was obtained from the difference between the mean of the weights of the alimentary tracts in the groups of unfed and fed roaches. It was realized that in the fed insects a part of the increased weight of the gut was probably due to accumulation of digestive secretions.

Salmonella *strains*

The *S. typhimurium* (strains 5609 and A-6087) and *S. montevideo* (strains B-33 and 5327) employed in these studies had been originally isolated from spray-dried egg powder (Solowey *et al.*, 1947), were lyophilized shortly afterwards and kept for several years in the dried state before being sent to our laboratory.* Both of these *Salmonella* types are known to cause human illness and to be harbored by various lower animals. It was decided to utilize two strains of each type in order to minimize the likelihood that a given set of results was due to unique attributes of a particular strain. Three of the 4 strains had demonstrated their ability to infect baby chicks when small numbers of viable organisms were administered orally (Milner and Shaffer, 1952).

To obtain inocula for the feces, each strain was seeded in tubes of infusion broth or slants of nutrient agar and incubated 24 hours at 37°C., the growth on solid medium being suspended in sterile saline solution. From these stock suspensions in broth or saline, further dilutions were made as desired in saline.

Preparation of artificially infected feces and enumeration of Salmonella *therein*

Samples of human feces were collected from two healthy donors known to be free of enteric animal parasites and pathogenic bacteria. To weighed portions of the stools, which were in most cases less than one hour old and never more than one day old before use, saline suspensions of *Salmonella* were added in the proportion of 3 parts to 20 parts of feces; they were thoroughly ground together in a mortar to give a mixture with consistency similar to that of freshly passed stool and with the *Salmonella* more or less uniformly distributed throughout. The inoculated feces were stored at 15°–25°C. in clean glass jars which were closed with rubber stoppers; the jars were kept on the laboratory bench exposed to diffuse daylight. There was little evidence of any striking differences in the ability of the various stool specimens to sustain the *Salmonella* introduced.

To estimate the number of *Salmonella* per gram of feces, a weighed sample was taken and from it serial decimal dilutions were made in saline; 0.1 ml. of each dilution was dispersed over the surface of an S S agar plate by means of a sterile glass "spreader" and the number of nonfermenting colonies counted after 48 hours' incubation at 37°C. Control platings were made from similar dilutions of samples of the same stools which had not been inoculated with *Salmonella*.

In addition, to permit estimation of the *Salmonella* even when the presence

* These strains of *Salmonella* were kindly furnished by Dr. Mathilda Solowey (Microbiology Research Division, Bureau of Agriculture and Industrial Chemistry, U. S. Department of Agriculture) to Dr. K. C. Milner who made them available to us.

of an excess of lactose-fermenting organisms prevented counting of the former on directly-spread S S plates or when the *Salmonella* inoculum was known to be less than 5×10^5 per gram of feces, a weighed sample of undiluted fecal material and dilutions thereof were seeded into tubes of tetrathionate broth and incubated for preliminary enrichment; loopfuls were subsequently plated on S S agar to isolate *Salmonella*. Their population in the feces was taken to be in the range of that number which would represent one *Salmonella* in the highest dilution yielding a positive cultural recovery. Thus if the organisms were isolated from inocula of 0.1 ml. of 10^{-1} to 10^{-3} dilution but not from seeding of 10^{-4} dilution, the number of *Salmonella* was estimated to be approximately 10,000 per gram of feces.

TABLE 1

Survival of Salmonella *in human feces kept at 15°–25° C.*

ORGANISM	RANGE OF NUMBERS OF *Salmonella* PER GRAM OF FECES			
	Initially present	After storage for		
		2 days	5–7 days	14 days
S. typhimurium (2 strains)	$1.9–3.1 \times 10^8$	$1.8–128 \times 10^8$	$.03–3.5 \times 10^8$	$0–1.0 \times 10^8$
	$0.8–4.0 \times 10^5$	$.03–1.0 \times 10^5$	$0–1.0 \times 10^5$	$0–2.0 \times 10^3$
	$1.0–1.8 \times 10^4$	$1.1–2.4 \times 10^3$	$0–8.0 \times 10^2$	$0–1.0 \times 10^2$
	$3.0–5.0 \times 10^3$	$1.1–1.3 \times 10^3$	$0–5.0 \times 10^2$	—
S. montevideo (2 strains)	$1.2–4.6 \times 10^8$	$1.1–28 \times 10^8$	$0.5–34 \times 10^8$	$0–0.35 \times 10^8$
	$0.4–3.0 \times 10^5$	$.001–1.0 \times 10^5$	$0–1.0 \times 10^4$	$0–1.0 \times 10^2$
	$1.0–1.5 \times 10^4$	$0.2–4.1 \times 10^3$	$0–0.1 \times 10^3$	—
	$1.0–2.0 \times 10^3$	$0.2–2.5 \times 10^3$	$0–0.1 \times 10^3$	—

EXPERIMENTAL DATA

Survival of Salmonella *in human feces*

The persistence of *Salmonella* in stored feces may be affected by diverse factors, including: a) the properties of the strain studied; b) the initial concentration of these organisms; c) the presence of competitive or antagonistic microbes; d) the physical and chemical properties of the feces; e) the environmental conditions to which the feces are exposed, e.g. temperature of storage, drying. In the present experiments attention was directed chiefly to the role of the first two factors mentioned. A total of 12 fecal specimens were inoculated with one or the other strain of *S. typhimurium*; 10 specimens were similarly seeded with *S. montevideo*. The results of attempts to recover *Salmonella* after varying periods of storage are summarized in Table 1.

In these trials, no strain showed a significantly greater ability to survive than the others; with each strain, *Salmonella* were recovered after at least 14 days in some experiments. Although it appeared that when the initial concentration of *Salmonella* was of the order of magnitude of 10^8 per gram of feces their population might actually increase during the first 48 hours of storage, the number of organisms in the seeding did not necessarily determine the

duration of survival. Thus in some trials with very large inocula the *Salmonella* were no longer recovered from the fecal specimens after 14 days' storage, whereas in 3 trials with initial concentrations of 5×10^4 or fewer *Salmonella* per gram of feces, the organisms were demonstrable after a fortnight or longer. In most trials, after 48 hours' storage the number of *Salmonella* recovered exceeded one per cent of the quantity initially present.

The presence of certain other micro-organisms was observed to influence the ability to recover *Salmonella*. With the appearance of moldy growth on the surface of the feces, for example, *Salmonella* disappeared. Likewise when *Pseudomonas* colonies predominated on the plates seeded directly with diluted fecal material, *Salmonella* were not isolated from such cultures nor from the tetrathionate broth enrichment procedure. It is possible that *Salmonella* were actually still present in the feces but they were overgrown or inhibited by *Pseudomonas*.

Quantity of feces ingested by P. americana *at a single feeding*

While the amounts of material eaten by roaches under natural conditions would be expected to vary (with the size and state of the animals, the quantity and kind of food currently available as well as that which they had ingested at the previous meal, the interval elapsed since the last feeding, etc.), it seemed desirable to obtain some approximation of the intake under the conditions of experiment. The weight of the intestinal tract was therefore determined for each of several animals all of which were starved for a week but some of which were allowed to feed on fresh fecal suspension immediately prior to dissection. Analysis of the values obtained for insects of similar size in the two groups revealed an increase in weight due to feeding which averaged approximately 0.1 gram ($P < .01$*). In view of the variation among the individual weights and the probability that a portion of any increment was due to the addition of digestive juices, it was considered that 0.1 gram approached the upper limit of the actual quantity of feces ingested and that some animals might have eaten as little as 0.02 gram. It may be pointed out that Olson and Rueger (1950) had no difficulty in feeding 0.1 ml., occasionally even 0.2 ml., of broth culture of *Salmonella* to adult *P. americana*.

Persistence of Salmonella *in cockroaches fed infected feces*

P. americana in which repeated prior examinations had revealed no fecal excretion of *Salmonella* were allowed to feed on human stools freshly inoculated with varying numbers of these bacteria and were sacrificed 2 days or 7 days later. The alimentary tract of each insect, including contents, was removed aseptically and separately ground with saline; the suspension was cultured for *Salmonella*. The findings are summarized in Table 2.

Most of the roaches which had ingested feces containing at least 10^5 *S. typhimurium* 5609 per gram were found to be harboring the organisms after 7 days

* Statistical examination of the data was kindly made by Dr. Hulda Bancroft of the Department of Tropical Medicine and Public Health.

as well as after 2 days. *S. montevideo* B-33 was likewise capable of persisting for the same periods in most roaches following ingestion in a concentration of 3×10^5 per gram of feces. With both of these strains only about 10 per cent of the roaches which were fed feces with $1\text{–}9 \times 10^4$ *Salmonella* per gram were later found to yield positive cultures from the gut and the frequency of isolation of the organisms was not significantly different whether the insects had been kept for the longer or the shorter interval of time.

No *Salmonella* were recovered from 19 roaches fed feces containing *S. montevideo* 5327 in concentration of 2.2×10^4 per gram or ten-fold greater. Similarly with *S. typhimurium* A-6087, of the 19 *P. americana* which had eaten feces with

TABLE 2

Persistence of Salmonella *in the alimentary tract of* Periplaneta americana *following ingestion of infected feces*

ORGANISM		LMONELLA	RATIO NO. POSITIVE TO NO. EXAMINED AFTER*	
Type	Strain	Per gram of feces fed	2 days	7 days
S. typhimurium	5609	1.8×10^7	6/8	4/4
		4.0×10^5	5/5	3/4
		1.0×10^5	5/5	3/5
		9.0×10^4	0/5	2/5
		1.2×10^4	1/5	0/5
		1.0×10^4	0/5	0/5
	A-6087	3.0×10^5	4/4	3/3
		4.6×10^4	0/5	0/5
		4.6×10^3	0/5	0/4
S. montevideo	B-33	3.0×10^5	4/4	4/4
		9.0×10^4	2/5	0/5
		1.5×10^4	0/5	0/5
	5327	2.2×10^5	0/5	0/5
		2.2×10^4	0/5	0/4

* Numerator = number of roaches from the intestinal tracts of which *Salmonella* of the type fed were recovered; denominator = number of roaches in the group, from which intestinal tracts were taken for culture.

less than 10^5 per gram none showed positive enteric cultures for these microorganisms although there was no difficulty in recovering them from insects receiving a very much larger dose.

DISCUSSION

The frequency with which roaches under natural conditions may have access to human feces containing *Salmonella* will depend on the prevalence of salmonellosis among the population, the lack of facilities available for the sanitary disposal of excreta and the length of survival of the *Salmonella* in the deposited feces as well as the number of the insects in the neighborhood. Our data, which conform to the general pattern of the information in the older literature concerning the survival of enteric pathogens in human feces (*e.g.* Kligler, 1921),

show that *S. montevideo* and *S. typhimurium* can persist in artificially infected stools kept in closed containers during periods of storage at 15°–25°C. up to 2 weeks or longer. In a few of our tests, not described in detail above, the rate of disappearance of the *Salmonella* was observed to be more rapid when drying of the feces occurred as a result of merely covering the mouth of the container with a double layer of gauze instead of using a tight-fitting rubber stopper. In any case, it appears that stools from a patient with acute salmonellosis might continue to serve as a source of infection if they were ingested by roaches even many hours after deposition.

It has been repeatedly observed that roaches readily eat human feces without the need for preliminary starvation; in certain supplementary trials carried out during the early phases of this investigation feces were used successfully as bait in traps, attracting not only *P. americana* but also *Blattella germanica* and *Supella supellectilium*. In our experiments with roaches which were starved for a week in order to provide opportunity to demonstrate that they were not excreting *Salmonella* in their feces as the result of prior exposure, it was uncommon to recover the organisms after feeding of feces containing less than $1–3 \times 10^5$ *Salmonella* per gram. While *S. typhimurium* 5609 could be recovered from a single *Periplaneta* which 2 days previously had fed upon material containing as few as 1.2×10^4 per gram, most animals exposed to similar or somewhat larger doses of any of the four bacterial strains proved refractory. If the roaches ate 0.02–0.1 gram of feces it may be concluded that for *S. typhimurium* 5609 and *S. montevideo* B-33 an intake of at least several thousand viables was necessary to ensure their persistence with a fair degree of regularity. We consider our figures to be in good agreement with those reported by Wedberg *et al.* (1949) as the minimum infective dosage of *S. typhimurium* for *Blaberus cranifer*.

Olson and Rueger (1950) found *S. oranienburg* could remain viable on the external body surface of *P. americana* for as long as 78 days. In the light of their observations and the fact that the method we used (enrichment in tetrathionate broth followed by plating on S S agar) had been shown to be capable of demonstrating the presence of a single viable *Salmonella* in a sample, our failure to recover these organisms from the intestinal tracts of roaches which in the process of feeding had crawled on feces containing thousands of *Salmonella* per gram may be taken as evidence that the technic of removing the gut for culture was bacteriologically adequate.

It appears that although certain strains of *Salmonella* may persist for at least a week in the alimentary tract of adult *P. americana* if the initial dosage is sufficiently great, this animal species may not be extremely susceptible since in our experiments an inoculum of approximately 1000 microorganisms failed to survive in most insects and some *Salmonella* strains were rapidly eliminated even when larger doses were used. By contrast, it has been shown (Milner and Shaffer, 1952) that a significant proportion of day-old chicks can be infected orally by as few as 20 viable *S. montevideo* B-33 or *S. typhimurium* strains 5609 and A-6087. From the foregoing, as well as the observation that when feces containing *Salmonella* were stored in vitro a considerable fraction of the number of organisms initially introduced could in most instances be readily recovered

for at least 2 days, frequently for longer periods, and bearing in mind the demonstrated ability of the cultural method employed to detect the presence of very small numbers of viable *Salmonella*, one is led to agree with the conclusions of earlier workers (e.g. Toda, 1923; Wedberg *et al.*, 1951) that there may be in the insect gut a mechanism other than mere movement within the alimentary tract and eventual defecation which is capable of eliminating moderate or even large numbers of certain *Salmonella* strains, especially *S. typhosa*. This mechanism may fail when overburdened by a massive dose of some organisms, permitting the latter to survive and induce infection. The recently reported experiments of Janssen and Wedberg (1952), indicating that *Blattella germanica* were capable of completely eliminating millions of *S. typhosa* within 12 to 18 hours after ingestion whereas *S. typhimurium* similarly administered were able to persist in the host up to 7 days, emphasize the problem, although these investigators were unable to account for the difference in behavior. It would be of considerable interest to ascertain the reason for the observed resistance of the cockroach to infection with various *Salmonella*, whether this be due to a non-specific physiological response or the result of an immunologic reaction.

SUMMARY AND CONCLUSIONS

1. Feces from healthy human donors were inoculated with varying doses of *S. typhimurium* (2 strains) or *S. montevideo* (2 strains) and the survival of the organisms during storage in closed containers at 15°–25°C. was determined. An appreciable proportion of the number introduced could be recovered in all trials after 2 days, frequently after 5–7 days, sometimes even after 14 days.

2. Adult *P. americana* which had been starved for a week were allowed to feed on uninfected human feces; the quantity ingested in a single meal was estimated to be approximately 0.02–0.1 gram.

3. Similarly starved *P. americana* were allowed to feed on human feces to which varying numbers of *Salmonella* had been recently added; after an interval of 2 or 7 days, the alimentary tracts were removed and cultured for the presence of these organisms. Survival in the gut was demonstrated to occur fairly regularly and to persist for at least 7 days when the insects had ingested in feces approximately 10^4 or more viable *S. typhimurium* (strain 5609) or *S. montevideo* (strain B-33). With smaller doses, recovery of the *Salmonella* fed was obtained only rarely. The limited data obtained with a second strain of *S. montevideo* (5327) suggested that it had somewhat less ability to persist than strain B-33.

4. Although the cockroach *Periplaneta americana* appears to have some mechanism for eliminating many hundreds of ingested *Salmonella*, if the insect eats feces containing at least several thousand of these microorganisms it may thereafter continue to harbor certain strains for at least a week.

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THE NAME "LEPROSY"

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I. INTRODUCTION

A problem that is unique in modern medicine results from the application of the Biblical name "leprosy" to an ailment which has been defined only during the lifetime of many physicians still vigorously at work. In no other disease is nomenclature a major hazard for public health. In no other disease is nomenclature a more serious barrier to sound medical care than any difficulty in diagnosis or treatment. An analogy might be imagined if Addison's Disease, with its characteristic dark pigmentation of the skin, had by some caprice of medical history been named "the Black Death." Even this analogy is extremely feeble: the Bible carries vastly more authority than Boccaccio!

My first realization of the horror associated with this word came in the year 1934. At a large medical center, I saw a woman who came for diagnosis of a nodular skin eruption. She had lived for a number of years in the tropics and had been told that she had "leprosy." An exhaustive examination, however, revealed a cancer with multiple metastases to the skin. When this condition was explained to the patient her response was unforgettable: "Thank God it's nothing but cancer!" (If I am a fanatic, I became so at that moment!)

Frequent references, both in Congress and in periodicals of high repute, to "the leprosy of Communism" and to "moral leprosy" reveal that the horror of the Biblical word is deeply graven in our language and was not merely a morbid obsession on the part of this patient. The medieval rigor of our Federal statutes as well as numerous court decisions emphasize the same feeling. I quote a typical example from a northern State. In 122 Iowa 651 the Court of Appeals remarked: "A charge of crime, in the ordinary sense of the word, is a mild and harmless imputation, when compared with words which brand a man or woman as a leprous outcast."! Iowa is one of the states where the ailment now called "leprosy" has never been known to be acquired. Hence the court was clearly referring to the *name* rather than to the *nature* of the modern disease.

The recent law in the Hawaiian Islands (1949) which makes official only the term "Hansen's Disease," is motivated by recognition of the paralyzing effect of the Biblical connotations of "leprosy" upon public health measures and also upon the application of effective treatment.

A desire to change the official name in order to avoid confusion with the Bible does not spring from sentimentalism, but from the hard fact that even today many patients who are told that they have "leprosy" change their names and go into hiding. They forfeit treatment for themselves in order to protect their families from ostracism. Even after a medical discharge from the U. S. Public Health Service Hospital at Carville, Louisiana, patients find it almost impossible to obtain employment, especially in northern towns where neither the physicians nor the public has even seen a patient with this disease.

PLATE I (a) EXAMPLES OF GREEK WORDS RELATED TO "LEPRA"
(Verbs, nouns, and adjectives are given in their simplest form)

The Biblical word "lepra", λέπρα is one of a large family of words coming from the root of the verb λέπω meaning to husk, to thresh, to scale, or to remove the bark. From this root two groups of words were derived, with almost opposite meanings. Those with the root λεπρ- carried the connotation "rough or harsh like a scaly surface". Those with the root λεπ- carried the connotation "thin, delicate, or fine like a scale". Hebrew, Greek, Latin, and English all have pairs of words in which opposite meanings depend upon a single consonant sound. "Thick" and "thin" in English are an instructive example.

ἔλεψα – 1. To peel off (a sheath). Homer's Iliad, book I, line 236. (960 B.C. ?) (aorist tense)

λεπτός – 2. Thin, frail. Hesiod (800 B.C.)

λεπιδωτός - 3. Covered with scales, as a crocodile. Herodotus (450 B.C.)

λεπίς — 4. The scales of a fish. Aristotle (350 B.C.)

λέπρα 5. A rough and scaling condition, applied to the bark of a tree. Theophrastus (325 B.C.)

λεπράς 6. Rugged and rough, applied to a rock. Theocritus the poet (280 B.C.)

λεπίς 7. The scales of a serpent. Nicander (160 B.C.)

λεπτόν 8. A coin so small and thin as to resemble a scale -- translated in the Bible as "the widow's mite". (Luke 21:2) (Also Mark 12:42) (Also Luke 12:59)

λεπίς 9. Flakes that fly from an anvil. Discorides the Naturalist (100 A.D.)

λέπος 10. Husks or scales. Pollux the Antequarian (180 A.D.)

λεπράς 11. An unpolished stone. Oppian the poet (190 A.D.)

λέπρα 12. (Attic and Biblical Greek) A chronic harmless skin eruption manifested by patches of dry scales. The Hippocratic Writings (400 B.C.) Galen (180 A.D.) Paulus Aegineta (650 A.D.)

λέπρη 13. (Also in the Ionic Greek)

λώβη 14. "Leprosy" -- as the term was used in Byzantine ecclesiastical law. Philes the poet (1300 A.D.)

PLATE I (b) HEBREW AND GREEK WORDS REFERRED TO IN THE TEXT

Hebrew

1. "Zaraath", translated as "leprosy" - צָרַעַת
2. "Zirah", translated as "hornet" - צִרְעָה
3. "Nega", translated as "stroke" or "plague" - נֶגַע
4. "Naga", translated as "to strike" or "to plague" - נָגַע
5. "Laben", translated as "white" - לָבֵן

Greek

1. To heal or to cure ἰάομαι
2. To cleanse καθαρίζω

The burden of proof is upon one who advocates a change in official nomenclature. The purpose of this article is to examine the evidence as to whether the Biblical name is *accurate*, regardless of its disastrous emotional associations. I will not attempt to disguise my own opinions, however; to pretend that I have the detachment of a computing machine would be both dishonest and ridiculous!

II. HISTORICAL SUMMARY

The word "unclean" as used in the Ritualistic Law is difficult for the modern mind to comprehend. It is commonly rationalized in terms of hygiene, or of morality, or of need for soap-and-water! Leviticus 11 and 12, and Leviticus 21:16 to 22:9 should make it clear that the words "taboo" or "stigma" are closer to the original meaning. Frazer's *Golden Bough* and the many studies which followed it show that "taboo" and "stigma" are universal in the thinking of primitive peoples.

The word "blemish" appears twenty-eight times in Leviticus. Examination of the text can hardly fail to impress the reader with the intensity of the "blemish-taboo." Protestant, Catholic, and Jewish scholars are in remarkable agreement as to the Biblical word "leprosy": it was not a medical term designating "Hansen's Disease" or any other specific disease. Rather it denoted an inflicted stigma or blemish which marked the victim as "unclean" under the Hebrew ritualistic law. It is illuminating to recall that the many million "untouchables" or "outcasts" of India are similarly regarded, and that neither hygiene nor morality nor soap-and-water has anything to do with their status.

In interpreting ancient texts, several hazards must be remembered. It is important to be aware that references to the Ebers Papyrus, to the early Sanskrit hymns, and to the Chinese of the Ming Dynasty, are fashionable ways of decorating an article with "quack scholarship." The Ebers Papyrus is an instructive example. The Egyptian hieroglyphics began to be deciphered with the discovery of the Rosetta Stone. From the few words which could be interpreted by comparison with the Greek portion of the Stone, our knowledge has been extended by the discovery of other fragments, especially those containing the names of kings, battles, or nations, which could be compared with records in other languages. Further, it is often possible to make a reasonable guess at the meaning of a word which is found in several inscriptions when the remaining words are known. Nevertheless, the names of *diseases* and of forms of *medication* are, even now, little more than a pyramid of hypotheses.

H. Joachim in the year 1890 audaciously published an attempt to translate the Ebers Papyrus into German. On page 119 of this "prescription book" he refers to "lepra?", and interprets the hieroglyphics as recommending onions and sea salt boiled in urine. A footnote appended to "lepra?" states that it is based upon Stern's conjecture "morbus leprosus?". A less technical translation into English, from the German of H. Joachim, was published by Cyril Phillips Bryan in 1931 under the title "The Papyrus Ebers." The question marks and sources are left out of the text, but a lengthy introductory chapter emphasizes the uncertainty of every disease and form of medication mentioned.

During the past twenty years a discussion of "leprosy" has usually included the remark that "leprosy was described in the Ebers Papyrus in 1500 B.C." The source for this dogmatic statement is not given. Yet it is made with a manner of final authority which the same physician does not use even when he describes the observations which he has made in his own laboratory.

There are other difficulties, however, far more subtle than fraudulent erudition. One is the change of any living language with time. The classical Greek of 400 B.C. was importantly different from the Homeric Greek of 950 B.C. and likewise from the Biblical Greek of the first century A.D. The Latin of the Golden Age was different from the Latin of the Vulgate four centuries later. The Hebrew at the time of the translation of the Septuagint (150 B.C.) had changed from the Hebrew of Nehemiah and Ezra, and inevitably underwent further change before the Massoretic text was crystallized.

Finally, the Hebrew consonant points and vowel markings were absent at the time of Jerome (400 A.D.), but are present in our oldest Hebrew manuscript of the Books of the Law (950 A.D.). The oldest Greek texts of the Bible, the Codex Vaticanus, Codex Sinaiticus, Codex Alexandrinus, and Codex Bezae, are written on parchment, which was very expensive. For this reason, punctuation, divisions between words, and accent marks are either absent, or were introduced by a later annotator (as is apparently the case in the Codex Vaticanus).

Nothing is definitely established regarding the etymology of the Hebrew word translated as "leprosy", "*zaraath*." If we remove the vowel markings, the similarity between this word and the word translated as "hornet", "*zirah*", is suggestive of a close connection, especially in view of the strange way the word "hornet" is used in the three places where it appears in the Bible (Exodus 23:28, Deuteronomy 7:20, Joshua 24:12). This amazing usage suggests a phrase of which the original meaning had been forgotten long before the description of leprosy in Leviticus 13 and 14. The Talmudic scholars, however, explained "*zaraath*" as the older of the two words. They wrote, of course, at a time when etymology was mostly imagination and allegory; it did not become a science until more than a thousand years later.

The repeated expression, "the plague of leprosy" is helpful. The noun used for plague, "*nega*" and the verb "*naga*" had a double significance which is not reflected in our English translation. The words were used to designate a stroke or blow inflicted from God. The word used in the King James translation of Isaiah 53:4, "yet we did esteem him *stricken*, smitten of God. . . ." is the same word which is used regarding the ten plagues of Egypt, Exodus 11:1, "Yet will I bring one *plague* more upon Pharoah. . . ." This is significant because everyone who is described in the Old Testament as contracting "leprosy" was made leprous because of an impious act.

Leviticus 13 and 14 demonstrate to the most skeptical that the word which is translated as leprosy did not apply to the condition officially called "leprosy" at the present day, and indeed did not define a disease entity at all. Only one of the six or seven conditions which are described can be recognized with reasonable assurance. This exception applies to the description of leprosy of the scalp with

the presence of yellow hairs (Leviticus 13:29–36). The identity with ringworm of the scalp (favus) is fairly plausible. Modern "leprosy" is extremely unlikely to involve the scalp.

The repeated instruction in doubtful cases to observe the patient for seven days, and then pronounce the condition leprosy if it had extended, indicates a rapidity of spread which is the opposite of the behavior of modern "leprosy." The absence of any reference to loss of sensation of the skin, or to wasting of the fingers or toes, or to blindness, is an omission of the most specific gross manifestations in untreated patients. The recurring expression, "leprous as snow" does not accord with the usual manifestation in the white race. Typically there is an increased pigmentation, and no loss of pigment except following ulcerative lesions. The phrase "leprous as snow" would be highly appropriate for psoriasis, and equally inappropriate for the disease which is at present called "leprosy." The "leprosy of garments" and "leprosy of houses" can apply to incrustations produced by molds and fungi, but never to what we now call "leprosy."

The Jewish scholars who translated the Old Testament into Greek (the Septuagint 200–100 B.C.) were confronted here, as in numerous other passages, with the problem of translating words from the Hebrew ritualistic law which had no Greek equivalent. The choice of the word "*lepra*", which was vaguely applied to scaly skin eruptions, was apparently intentional in order to avoid confusion with any well-recognized entity. It is otherwise difficult to explain why "*lepra*" (meaning a scaly condition) should have been chosen when scaling is one feature absent from the descriptions in the Bible, with the single exception mentioned as ringworm of the scalp. (The Hebrew word used for "white", "*laben*", resembles the Greek word for a white scaling eruption, "*lepra*", especially if we confine our attention to the Greek and Hebrew biconsonant roots. But that this may have suggested a false etymologic relationship to the translators of the Septuagint seems preposterous.)

The writers of the New Testament followed the example of the Septuagint. One passage in the New Testament is of supreme importance in indicating that the word was regarded strictly as a part of the ritualistic law. This is the passage in Luke which relates the cleansing of the ten lepers. In every other instance in the New Testament, the verb used with diseases is "heal" and with leprosy is "cleanse." In the case of *one* of the ten lepers, however, the verb "to heal" is used (Luke 17:15). This one man was a Samaritan. Therefore, he could, by nature, never be "cleansed" in the technical sense of the Jewish ritual.

The exception in speaking of the Samaritan is paralleled in the case of Naaman (II Kings 5). Naaman was a Syrian, hence we need not be surprised that his condition did not bar him from being commander of the Syrian Army instead of an outcast. The restrictions of the ritualistic law did not apply to him. According to the Jewish Encyclopedia, the writers of the Talmud likewise held that the law did not apply to a person who was not Jewish.

The translation of Luke 17:15 into the Latin Vulgate by St. Jerome (400 A.D.) has been the object of unjust criticism which even a Protestant like myself must point out. The Latin which is commonly taught in school is that of the "Golden

Age." Caesar and Cicero, however, are approximately as distant in time from St. Jerome as Chaucer from Churchill! The Latin word in Luke 17:15, which is translated as "heal" (King James Version) or as "cure" (Knox Version), is the passive of the verb "*mundo*" in the Vulgate. The ordinary Latin dictionary defines this verb as meaning "cleanse", which indeed was the word used in translating Luke 17:15 in both the Protestant versions before 1611, and also in the Catholic Douai version (1609).

Latin, however, is a language in which verbs tended to be few and generic. The nouns were depended upon for vivid and specific meanings. The quotations which I have been able to discover in the Latin of the time of St. Jerome indicate that the verb "*mundo*" had a much broader meaning than "cleanse." The English verb "clear" or "clear up" excellently represents this broad meaning. We speak of rubbish as being "cleared away", or of a skin eruption as "clearing up", or of a meaning as being "made clear." Similarly, Jerome could with accuracy use the familiar "*mundo*" with "*lepra*" (Luke 17:15) in order to avoid excessive emphasis on a technical distinction which had become nearly obsolete by his time. He could with equal accuracy depart from his ordinary usage and employ "*curo*" with "*lepra*" to emphasize an exception in Naaman, who was a general from a hostile and idolatrous country (Note II Kings 5:1–7)

Regarding the confusion between "*lepra*" and "*elephantiasis*", I can speak with personal experience only of the vigorous and colorful Greek text of Paulus Aegineta. This seems to me a complete jumble. "*Lepra*" definitely includes psoriasis, dry eczema, dry ringworm, probably even dandruff. "*Elephantiasis*" evidently confused mycosis fungoides, lupus vulgaris, pemphigus vegetans, blastomycosis of the skin, and apparently even von Rechlinghausen's disease, and syringomyelia. The first group ("*lepra*") is chronic, scaling, and harmless; the second group ("*elephantiasis*") is chronic, non-scaling, and destructive.

During the Middle Ages, according to the Catholic Encyclopedia, persons pronounced as suffering from "leprosy" had the funeral rites of the dead performed for them. Their property was usually confiscated, and they were excluded from inhabited towns. The Catholic Encyclopedia further quotes Matthew Paris as stating that there were 19,000 leprosaria in western Europe in the 11th century. It adds, however, that a large number of conditions were doubtless diagnosed as "leprosy" without being what would be called "leprosy" at the present day. Between about 400 A.D. and 1400 A.D. there are almost a thousand years with very few descriptive records of any value that I have been able to discover. In the *Oxford Medicine*, (V, Chapter 27-C), E. H. Hudson has reviewed the medieval sources, with the conclusion that what was called "leprosy" was largely syphilis (as yet unrecognized as a separate disease).

It is of considerable importance that the diagnosis of "leprosy" almost completely disappeared from western Europe with the great "epidemic of syphilis" that began about 1493. The 11th edition of the Encyclopaedia Britannica (1911, Volume XVI, page 480) makes the naive statement: "During the 15th Century the disease ('leprosy') underwent a remarkable diminution. It practically disap-

peared in the civilized parts of Europe, and the leper houses were given up. It is a singular fact that this diminution was coincident with the great extension of syphilis."

In view of the difficulty of diagnosing skin eruptions even at the present day, we have scant justification for asserting that there was a change in the *frequency of a disease* in the Middle Ages simply because there was a change in the *frequency of a diagnosis*. The descriptions of syphilis (1493–1530) are numerous and detailed. They suggest yaws, or else the destructive type of secondary syphilis. In any case, "syphilis" afforded a convenient way of explaining any skin eruption without making the patient an outcast. If the patient was of the nobility or a man of wealth, the change in nomenclature to "syphilis" must have been profitable to the physician as well! This may explain in part the belief that syphilis affected mostly persons of high rank, a belief that made "the noble disease" one of the legion of synonyms for syphilis, and that explains why the poem which gave syphilis its present name could be piously dedicated to a Cardinal of the Church.

It has been stated that the frequency of the diagnosis of syphilis in the courts of various kings was responsible for the term "courtplaster", which was first used to cover the skin lesions of syphilis, and later (cut into artistic shapes) was used as "beauty marks", and finally became an adhesive bandage still used in some parts of our own country. I am at present, however, unable to find the original source of this interesting story.

The statement that the epidemic of "the Black Death" (plague) exterminated almost all patients with "leprosy" in Europe during the Middle Ages cannot apply to what is now termed "leprosy": the two diseases at the present time have their greatest frequency in the same places, especially in China, India, and Africa. One might more logically argue that they are not competitors but rather conspirators!

In Europe, the use of the word "leprosy" lingered in several scattered areas. It apparently was applied to one of several ailments, each found in a narrowly limited area. One of these peculiar localized diseases was observed in Iceland and a few places in the Scandinavian peninsula. There it was first adequately described by Danielssen and Boeck in 1848, and specifically defined by the publication in 1874 of Hansen's microscopic studies. Only since that time has the word had its *present official meaning*.

The condition which is conveniently referred to as Hansen's Disease (in order to avoid confusion with the Biblical terminology) has a remarkable distribution. It is estimated to affect a total of five million people, most of whom inhabit the tropical zone. The mystery of its transmission is evident when one considers the fact that the microscopic rod, which resembles the tubercle bacillus and which is characteristic of the disease, will not grow on culture media or infect laboratory animals. In more than one hundred attempts it has failed to infect human volunteers. No physician or nurse at the Carville Hospital has contracted the disease since the institution was founded in 1894. Finally, though about one hundred

and seventy-five persons with the ailment came to northern Michigan, Wisconsin, and Minnesota during the latter part of the 19th century, the disease died out in those states despite the most intimate contact in early childhood. The very limited distribution of Hansen's Disease in the extreme tip of Florida, a small area in Louisiana, and a small area in Texas is difficult to explain. Why a tropical disease should be found in a few small areas in the extreme north of Europe is still more puzzling. It is at least a plausible theory that the organism must pass part of its life cycle in some insect or other host between one human infection and another. The disease has never been proved to be contagious—indeed, the acid-fast microscopic rods have never been *proved* to be living organisms!

An attempt to explain the etiology of Hansen's Disease, however, is not the purpose of the present study. In 1941 Dr. Faget and Dr. Johansen at Carville first observed the dramatic effect of promin upon the ailment, and since that date chemical derivatives of promin have been synthesized which are even more effective, less toxic, and easier to administer. Only since that date have we had a specific and reliable treatment. The central problem today is not the discovery of a more effective medication. Rather the problem of today is the removal of a barricade between patient and treatment created by a disastrous nomenclature.

The *incorrect* use of the Biblical word "leprosy" creates a curtain of terror. Such is the tragedy of the disease that now bears this name. The original texts leave no reasonable doubt that the Hebrew "zaraath", the Greek "lepra", and modern "leprosy" have three entirely unrelated meanings. The naming of a disease is ordinarily thought of as void of human interest except in profitless academic disputes. For five million of the sons of men with this ailment, however, the verse "A good name is better than precious ointment"* has immediate and terrible truth!

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Appendix (A): Leprosy in the Bible

Though the words "leper", "lepers", "leprosy", or "leprous" are found sixty-eight times in the King James Bible, they occur in a relatively small number of passages. The words used in the Hebrew of the Old Testament are always "*zaraath*" or a related form, and in the New Testament "*lepra*" or a related form.

* Ecclesiastes 7:1

Exodus 4:6	Moses receives power to make his hand leprous and to restore it, as a sign to Pharoah.
Leviticus 13 & 14	Detailed ritualistic law regarding the leprosy of persons, of garments, and of houses.
Leviticus 21:16 to 22:9	Leprosy listed among the blemishes that would bar a descendant of Aaron from serving as priest.
Numbers 5:2	Lepers listed among those excluded from the camp.
Numbers 12:10	Miriam made leprous for seven days for speaking against Moses.
Deuteronomy 24:8	Warning to remember the punishment of Miriam.
II Samuel 3:29	David's curse on Joab for murder.
II Kings 5	Elisha cures Naaman's leprosy, but Gehzai is cursed with leprosy for his avarice.
II Kings 7:3–16	Four lepers excluded from camp during siege, and their adventure.
II Kings 15:1–7	King Azariah made leprous for impiety.
II Chronicles 26	King Uzziah made leprous for impiety. (A fuller account of the same story as given in II Kings 15:1–7).
Matthew 8:2–3 Mark 1:40–42 Luke 5:12–13	Jesus cleanses a leper.
Matthew 11:5 Luke 7:22	Cleansing told to the disciples of John.
Matthew 26:6 Mark 14:3	"The house of Simon the leper" is mentioned.
Matthew 10:8	The disciples told to heal the sick and cleanse the lepers.
Luke 4:27	The story of Naaman mentioned.
Luke 17:12–19	Ten lepers cleansed.

It is interesting to note that the word is never used in the Prophets or Psalms, nor in the Acts or Epistles.

Appendix (B): References to *LEPRA* in the Hippocratic Writings

More than 70 books have been attributed to Hippocrates. The variety of dialects, dates, styles, and opinions, however, makes it evident that this library includes many independent authors. It is impossible to prove that Hippocrates wrote any of the books in the collection, or even that the shadowy "Father of Medicine" ever existed.

The Encyclopaedia Britannica, (1947) Volume XI, Page 583 B, refers to the story that Hippocrates was descended from Asclepios, and makes the amazing remark: "Since Asclepios is an historical figure—being mentioned as a physician in the *Iliad*—(this) is at least possible"! The same evidence would make Zeus, Poseidon, Apollo and Aphrodite "historical figures"—they are also mentioned in the *Iliad*!

Greek mythology relates that Asclepios was a son of Apollo and inherited his father's miraculous powers of healing. The myth which describes the act that caused Asclepios to be elevated to an Olympian god is worthy of comment. The horses of Hippolytus were terrified by the god Poseidon, and Hippolytus was trampled to death. Asclepios restored him to life. As a result, Zeus (after some factional strife on Mount Olympus) made Asclepios a god. The manner of death of Hippolytus and his restoration to life by Asclepios make the Greek name "Hippocrates" very significant. A physician, regarded as descended from As-

clepios and as possessing the healing art of his divine ancestor, might appropriately receive this title, because the Greek word "Hippocrates" means "*master, ruler, or conqueror of horses.*"

This suggests that the word "Hippocrates" was originally a metaphor applied to the physician who had exceptional power in healing, and that this figure of speech was later mistaken for a personal name. My suggestion imposes some strain upon human credulity, but is hardly as preposterous as the quotation which I have given from the encyclopedia!

There are two books in the collection attributed to "Hippocrates" which contain the word "*lepra.*" The word is used in two passages in the book on *Nutriment* which has an oracular style and often becomes entirely meaningless. More important is the *Prorrhetic II* which is now generally regarded as a very early source used in several of the later writings.

I translate the Greek of the Loeb Classical text, Harvard University Press, 1939, in quoting from sections XVII and XX of the *Nutriment.* I have departed from W. H. S. Jones in translating several medical words. The Cambridge scholar knows Greek, but understandably lapses at times from modern English medical terminology. These paragraphs show the book on *Nutriment* at its worst: "No subject—no predicate—and no meaning."

XVII. "Secretions in accord with nature, by the bowels, urine, sweat, sputum, mucus, menses; through hemorrhoid, wart, '*lepra*', tumour, carcinoma; from nostrils, lungs, bowels, perineum, penis; in accord with nature or contrary to nature. The peculiar differences in these things depend on differences in the individual in the time and in the manner.". . . .

XX. "Ulceration, eschar, blood, pus, lymph, '*lepra*', scurf, dandruff, lichen, leukoderma, freckles, sometimes harm, sometimes help, sometimes neither harm nor help."

The *Prorrhetic II*, last paragraph, I translate from the Greek text of Littré, with one important emendation which will be explained shortly. The Greek is ambiguous in several places, and twelve variant readings which are listed by Littré add to this ambiguity. I have attempted to reproduce the identical ambiguity in translating. (A clear and harmonious sentence structure would seriously misrepresent the chaotic state of the Greek texts!)

"Lichen, '*lepra*', and leukoderma: When these conditions begin in childhood or infancy, or after beginning very small, enlarge little by little for a long time, it is necessary to think of such eruption not as a discharge but as a disease. Yet these are a discharge when the eruptions arise abruptly and widespread. Leukoderma develops among the most fatal conditions like the disease called consumption. But '*lepra*' and lichen arise from black bile. They are more easily cured, the younger the patient, the earlier the stage of the ailment, and when they appear in the soft and fleshy parts of the body."

I have departed from the Greek of Littré in restoring the phrase "*the disease called consumption*". "Consumption" appears in the most ancient manuscripts and is frequently used in the Hippocratic writings. The expression, "*the disease called Phoenician*", does not occur elsewhere in the Hippocratic writings, and would in any case be impossible to understand. Littré rejected the oldest texts out of his veneration for a comment attributed to Galen.

A PRELIMINARY REPORT ON A "PERIODIC TENDENCY" OF MICROFILARIAE OF *WUCHERERIA BANCROFTI* OBSERVED IN TAHITI, FRENCH OCEANIA

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The so-called "nonperiodicity" of microfilariae in the peripheral blood of subjects infected with the South Pacific strain of *Wuchereria bancrofti* has been a subject of interest since the work of Bahr (1912). This has been so because in most other regions of the world where filariasis exists the microfilariae of *W. bancrofti* exhibit a marked nocturnal periodicity. Aside from this difference, there is little evidence that the parasite in the Pacific is not *W. bancrofti* and it is tentatively being considered as such in this paper.

Reports concerning positive or negative evidence of periodicity of microfilariae among South Pacific Islands vary considerably. Bahr (1912) reported that the filaria of Fiji was of the nonperiodic type. In the Philippines, Africa *et al.* (1935) found both the periodic and nonperiodic types, the former with greater frequency. Other investigators working in the Samoa, Ellice, and Wallis Islands (Buxton, 1927) have corroborated the finding of Bahr. O'Connor (1928), however, in studies in the Ellice Islands, found that there was a slight and inconsistent tendency for the numbers of microfilariae to recede between the hours of 2 a.m. (0200) and 7 a.m. (0700), but he concluded that the microfilariae did not show a typical rhythmic fluctuation in the peripheral blood. More recently Eyles *et al.* (1947), while studying the microfilarial densities of two subjects believed to have contracted filariasis in the South Pacific, one in the Society Islands and the other in Tonga, found that both subjects exhibited microfilarial peaks between noon and 8 p.m. Galliard *et al.* (1949), in making a limited study in Tahiti, found no evidence of microfilarial fluctuation during a 24-hour period.

During the present filariasis investigation in Tahiti, the microfilarial densities in the blood of a number of Tahitians were studied at different times of day in order to determine whether periodicity occurred in that area.

MATERIALS AND METHODS

The subjects, whose blood was studied in all tests, lived at widely distributed points around the island of Tahiti. They were for the most part normal, apparently healthy people. Groups studied included both sexes, their ages ranging from 7 to 69 years.

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All subjects in the first three trials came from one small community in the district of Papara. There, the technique of Brady and Lawton (1944) was used. This technique for counting microfilariae consisted of drawing accurately measured 20-cmm. samples of blood from a finger puncture. Each sample was diluted with one cc. of 0.1 N. hydrochloric acid, delivered into a Sedgwick-Rafter counting chamber and counts were made immediately. In using this technique, all samples were drawn by the second author and read immediately by the senior author. Several samples were examined by others to check for accuracy in counting.

In all other phases of the study, counts were made from thick blood films, each containing 20 cmm. of capillary blood. All specimens except those reported

TABLE 1

Numbers of microfilariae in a series of 20-cmm. blood samples from finger punctures of two subjects to show the variation in sampling. Tahiti, 1949

SUBJECT[1] NO. 1	SUBJECT[2] NO. 2
43	59
48	56
59	73
49	50
49	47
63	73
54	51
47	68
	44
	77
Total..... 412	598
Mean..... 51.5	59.8
Standard deviation.. 6.67	12.08

[1] Smears made between 1130 and 1210.
[2] Smears made between 1605 and 1650.

in Figure 2 were drawn by the authors. Films were dried for 4 to 24 hours, dehemoglobinized, dried again and then fixed for 5 minutes by immersion in 100 per cent methanol. All smears were stained with a 1:20 dilution of Giemsa for 15 to 20 minutes. It was found that this technique resulted in clear preparations in which microfilariae were easily detected and rendered suitable for minute morphological study. All smears were surveyed completely with the aid of an ocular guide or a Whipple disc. All microfilarial counts were made by the senior author and Madame Lamarquand, head technician.

RESULTS

At the outset, a brief study was made of the variation in microfilarial density which may be found in sampling. The data are presented in Table 1. A series of eight 20-cmm. smears was drawn from the fingers of one subject between the

TABLE 2

Numbers of microfilariae in 20-cmm. of capillary blood samples from 26 subjects at different times of day. Papara, Tahiti, March, 1949

Number of microfilariae

CASE NO.[1]	A.M.		P.M.	
	0145–0630	0745–0947	1313–1735	1930–2030
1	51	54	79	128
2	89	82	96	133
3	121	102	269	213
4	0	0	1	0
5	9	10	21	15
6	2	0	3	0
7	19	24	61	91
8	46	20	55	79
9	0	0	1	0
10	104	102	170	202
11	0	0	1	0
12	55	48	60	65
13	83	65	99	67
14	277	396	628	550
15	3	5	8	7
16	13	3	20	21
17	13	8	11	5
18	99	46	81	125
19	32	40	59	51
20	7	7	5	13
21	17	20	13	9
22	109	86	181	181
23	11	13	29	18
24	1	3	8	2
25	99	141	353	193
26	24	37	85	67

Analysis of variance (transformed data)

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	F RATIO	F RATIO REQUIRED @ 99 TO 1
Person differences	25	102.88	55.5[2]	2.07
Periods	3	30.47	16.6[2]	4.06
within A. M.	1	.28	.15 N.S.	6.98
within P. M.	1	1.60	.88 N.S.	6.98
A. M. versus P. M.	1	89.54	48.9[2]	6.98
Remainder (error)	75	1.84	—	—
Total	103	—	—	—

[1] Ages of subjects ranged from 20 to 52 years with the exception of number 26, a 9-year-old girl. Not listed are the results for 7 persons whose bloods were negative for microfilaria.

[2] Highly significant, $P < .01$.

N.S. not significant.

hours of 1120 and 1210, and 10 smears were made from a second subject between 1605 and 1650. The microfilarial counts of these patients ranged from 43 to 63 and from 44 to 77 respectively, with means of 51.5 and 59.8 and standard deviations of 6.67 and 12.08. In general, sampling variation can be expected to follow Poisson's distribution, "Student" (1907), and the standard deviation may be estimated as the square root of the arithmetic mean. For the two sets of data reported here, the expected standard deviations could thus be estimated as 7.17 and 7.73 respectively. In order to test for periodicity in the data to be presented below, it will be better, by statistical techniques, to account for the variation which may be expected from sample to sample, rather than to attempt to define a range beyond which differences in counts are to be considered significant. This

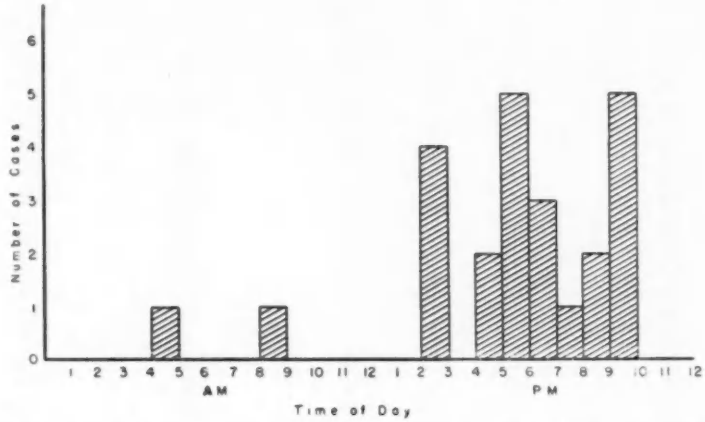

FIG. 1. Hours of the day when greatest numbers of microfilariae were seen in a series of 4 to 6 smears taken during a 24-hour period from 24 subjects. Tahiti, 1949.

is necessary because the ranges may vary widely depending on the magnitude of the counts.

In the first two periodicity studies, Trials I and II, 26 persons were examined. Counts of the microfilarial density were made on four specimens of blood drawn at intervals during a 24-hour period. The results are shown in Table 2 and Figure 1. It is of interest that 4 of the subjects were among 12 who had been negative in a pilot survey, but were found positive in at least one of the 4 smears; 3 of these were negative in morning smears but positive in the afternoon. It is to be noted that all but 2 of the cases had their greatest counts in the afternoon.

An analysis of variance was conducted on the data for the 26 individuals. For theoretical statistical considerations, the analysis was computed using the square roots of the counts, Snedecor (1946), rather than the actual counts. As a result of the analysis of variance technique, it was possible to remove the variation among individuals and to consider only "pure" variation from time period to time period. This consideration showed no significant differences in total microfilariae within the two a.m. periods, 0145–0630, compared with 0745–0947,

or within the two p.m. periods, 1313–1735 compared with 1930–2030. However, there was a substantial and highly significant ($P < .01$) difference between a.m. and p.m. levels. The abridged analysis of variance table is given in Table 2.

In Trial III an attempt was made to ascertain more definitely the time of day when the microfilariae are most and least numerous in the peripheral blood. Specimens were drawn four times between the hours of 0605 and 0950 and four times between 1800 and 2100. Seven of the subjects of Trial II were studied again in this manner; 5 of these had also been used in Trial I. The results are presented in Table 3. With the exception of subject number 23 at 2025, all persons exhibited more microfilariae in the evening than in the morning. With the exception of subject No. 2, the greatest numbers of microfilariae were present between 1830 and 1930.

TABLE 3

Numbers of microfilariae in 20-cmm. blood samples from 7 subjects at different hours of the day. Papara, Tahiti, 1949

CASE NO.*	MORNING				EVENING			
	0605–0710	0720–0755	0805–0847	0907–0950	1730–1825	1830–1924	1940–2025	2038–2100
	No. M/F	No. M/F	No. M/F	No. M/F	No. M/F	No. M/F	No. M/F	No. M/F
1	38	40	55	68	152	169	107	157
2	55	61	52	47	154	124	86	167
7	36	41	37	47	80	91	63	62
8	26	24	18	36	56	61	41	53
12	65	15	44	47	94	104	84	94
23	13	12	8	10	22	25	12	20
26	22	19	23	21	46	56	33	46

* Ages ranged from 9 to 52. There were four males and three females.

The data in Table 3 were analyzed by a technique that eliminated person-to-person differences without the calculations necessary for the analysis in Table 2. By ranking each time period within an individual (from 1 to 8, the highest number of microfilariae being given the score 1, the lowest the score 8) it was possible to obtain a set of coded scores for the 7 subjects listed in Table 3. By time period, this method gave the following scores:

Time	*Score*
0630	43
0730	45½
0830	47½
0930	44½
1800	16
1900	9
2000	28½
2030	18

Again, there is substantial evidence of an increase in the afternoon, with a possible peak at about 1900. There is no evidence of a morning trough for the three-and-one-half hour span studied.

To insure that this tendency of an evening influx of microfilariae in peripheral blood was not limited to the community studied in the first three trials, three other communities at widely separated points around the island were selected for study in Trial IV. Only one morning and one evening smear were made for

TABLE 4

Numbers of microfilariae in 20-cmm. blood samples taken morning and evening from subjects in 3 districts. Tahiti, 1949

GROUP A				GROUP B				GROUP C			
Tiarei—May 13, 1949				Mahina—May 13, 1949				Mataiea—May 14, 1949			
Case No.*	No. of M/F		Ratio 1	Case No.	No. of M/F		Ratio 1	Case No.	No. of M/F		Ratio 1
	a.m.	p.m.			a.m.	p.m.			a.m.	p.m.	
1	22	82	3.7	1	46	331	7.2	1	2	28	14.0
2	205	706	3.4	2	7	44	6.3	2	5	36	7.2
3	28	88	3.1	3	3	11	3.7	3	10	44	4.4
4	42	97	2.3	4	94	287	2.9	4	11	48	4.4
5	10	22	2.2	5	6	12	2.0	5	18	53	2.9
6	172	352	2.1	6	85	166	1.9	6	32	73	2.3
7	10	20	2.0	7	138	241	1.8	7	173	393	2.3
8	63	115	1.8	8	47	83	1.6	8	1	2	2.0
9	325	506	1.6	9	46	73	1.6	9	167	327	2.0
10	87	110	1.3	10	23	37	1.4	10	43	82	1.9
11	166	208	1.3	11	9	12	1.3	11	16	27	1.7
12	22	23	1.1	12	24	26	1.1	12	100	166	1.7
13	2	1	0.5	13	9	8	0.9	13	128	203	1.6
				14	11	9	0.7	14	36	53	1.5
				15	14	7	0.5	15	29	42	1.4
								16	80	80	1.0
								17	10	9	0.9
								18	42	36	0.9
								19	68	54	0.8
								20	5	3	0.6
								21	5	2	0.4
Smears made between 0643 to 0855 in the morning and between 1830 to 1923 in the evening. Ages 21 to 69.				Smears made between 0615 to 0828 in the morning and between 1820 to 1925 in the evening. Ages 22 to 56.				Smears made between 0636 to 0756 in the morning and between 1832 to 1947 in the evening. Ages 7 to 62.			

* Not shown in the table are the results for: 14 persons in Tiarei, 17 persons in Mahina, and 14 persons in Mataiea found negative for microfilariae, both morning and evening.

each subject. The results are shown in Table 4. Twelve of 13 persons in Group A, 12 of 15 in Group B, and 15 of 21 in Group C showed fewer microfilariae in the morning than in the evening. Thus a total of 37 of the 49 persons showed fewer microfilariae in the morning than in the evening. If all cases showing maximum densities of less than 15 microfilariae are eliminated from these results, only 2

of 39 persons did not exhibit more microfilariae in the evening than in the morning.

Five of the subjects exhibiting the highest differences between morning and evening counts were children who were selected from Trial IV, Group C, for further study to ascertain if the rhythmic fluctuation of microfilariae would be consistent over a long period of time. Their bloods were examined morning and evening (0700 and 1900) on six days over widely spaced intervals during one year except for subject No. 5 who was examined on four days. As shown in Figure 2, with one exception, i.e. number 3 in January 1950, all exhibited more microfilariae in the evening than in the morning.

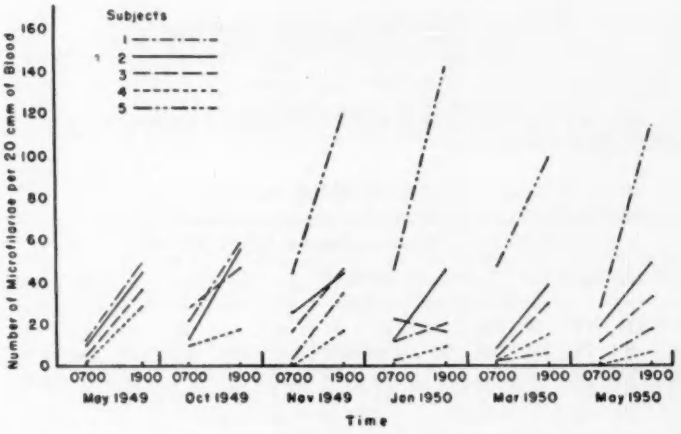

FIG. 2. Microfilariae per 20 cmm. of capillary blood from 5 children taken morning and evening on 6 days during one year. Tahiti, 1949–1950.

In addition to subjects whose microfilarial densities were measured in the aforementioned experiments, the blood of 19 others being used in vector experiments or examined on other occasions was studied at different hours of the day. With the exception of 2 persons, the microfilarial density was higher in the evening than in the morning. Opportunity for further study was afforded when one of the laboratory assistants, who had been negative for microfilariae several months earlier, became positive. A series of 40 20-cm. smears was made from his blood at different hours on several days over a period of two weeks. Results of microfilarial counts from only those smears taken around 7 or 8 a.m. and 7 or 8 p.m. are shown in Figure 3. Here again, there was evidence of a higher number of microfilariae present in the evening than in the morning.

As stated earlier, the question arose as to whether there might be a seasonal fluctuation in microfilarial density. Results of a single monthly examination of 25 positive persons during a period of one year are shown in Table 5. All of the individuals were examined at least eight times. These data were analyzed by

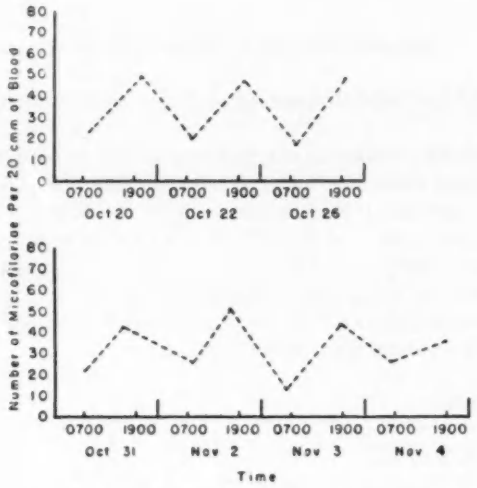

FIG. 3. Microfilariae per 20 cmm. of capillary blood from 1 subject taken morning and evening for 7 days. Tahiti, 1949.

TABLE 5

Numbers of microfilariae in 20-cmm. blood samples of capillary blood taken each month from subjects in Papara, Tahiti, 1949–1950

CASE NO.	SEX	AGE	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	AVERAGE NO. M/F
3	F	31	75	59	107	81	99	77	—	—	72	—	—	60	78.8
6[1]	M	8	72	168	300	230	186	381	417	225	343	413	268	398	283.4
7	M	9	0	0	6	17	36	78	110	79	113	96	58	39	52.7
8[1]	M	7	67	33	79	105	91	83	71	61	110	127	81	124	77.7
9[2]	M	11	0	0	0	0	0	0	0	0	0	1	—	0	0.1
11[1]	M	32	174	218	159	144	138	105	233	197	212	307	222	236	194.6
13[2]	F	11	0	2	0	1	0	2	3	1	6	1	—	5	1.9
20	M	17	0	0	1	2	0	0	0	0	0	0	2	6	0.9
24[1]	F	46	363	286	283	311	278	187	300	355	299	321	301	194	288.2
29	F	36	0	0	0	0	0	—	—	0	0	1	0	0	0.1
32	M	52	83	—	124	110	76	77	—	—	—	99	120	145	104.3
33[1]	M	49	12	25	19	14	18	44	19	16	23	28	20	24	21.9
36[1]	F	46	55	60	132	157	290	185	181	114	226	243	176	49	155.7
37[1]	M	20	26	27	21	39	23	36	50	37	24	20	59	33	32.9
38	M	22	0	0	0	0	0	0	0	0	0	0	1	0	0.1
39	M	29	1	—	1	5	6	7	8	4	12	11	6	8	6.3
40[1]	M	56	104	112	270	64	121	133	199	197	228	283	161	338	184.2
42[1]	F	29	30	29	35	23	22	26	15	18	20	15	8	11	21.0
44[2]	F	40	98	188	167	145	154	196	161	103	168	—	136	165	152.8
48[2]	M	45	526	515	707	597	—	632	558	438	262	405	356	290	480.5
49[2]	F	52	66	179	150	146	—	194	214	182	200	223	270	281	189.5
50	M	54	63	75	147	117	107	—	—	107	55	29	—	75	86.1
51[1]	F	27	42	52	75	64	85	87	78	88	59	95	99	81	75.4
52	F	11	43	—	26	36	—	90	21	14	12	—	24	34	33.3
57[2]	F	36	49	63	60	62	33	—	61	50	63	74	67	62	58.2

[1] Individuals with moderate to high counts for whom observations were made every month.

[2] Individuals with moderate to high counts for whom observations were made for 11 months.

the same ranking technique employed for the statistical evaluation of data in Table 3. Using only those 10 individuals for whom there were observations every month, (eliminating case No. 7, in whom it was suspected that there was an increasing microfilarial density during the period studied) the following scores were obtained by months:

April	87	Oct.	55
May	80	Nov.	69
June	70½	Dec.	59
July	73	Jan.	36½
Aug.	76	Feb.	56
Sept.	60	March	58

If the level in all months were roughly the same, one should have expected a total score of 65 each month. The scores found differ significantly ($P < .01$ using the Chi-square test) from the expected value of 65. It is not possible to determine whether this difference is due to a seasonal pattern (difference in seasonal rainfall) or whether there had been an increase in the level of infection of the individuals studied.

Performing the same analysis on the 6 additional individuals on whom 11 observations had been made (and making proper allowance for estimating the rank of the month for which no observation had been made) leads to an almost identical conclusion. December is the peak month for this group, compared with January for the others. April is the low month for both.

DISCUSSION

The results of the study reported here are in agreement with the findings of Eyles *et al.* (1947), that there is evidence of "microfilarial periodicity" in subjects of the South Pacific Area. Ishmeli in Fiji (pers. commun.) and Jachowski in American Samoa (pers. commun.) have indicated that they also have found evidence of microfilarial periodicity among subjects of those areas. It therefore appears probable that a rhythmic fluctuation of numbers of microfilariae in peripheral blood is characteristic of filariasis of several South Pacific Islands and for the present the proposal of "diurnal periodicity" suggested by Eyles *et al.* (1947), would seem to be a more appropriate name for the South Pacific strain of filariasis than the term "nonperiodic."

It is also apparent that the fluctuation in numbers of microfilariae in peripheral blood between morning and evening does not reach the pronounced day and night proportions seen for *W. bancrofti* in other parts of the world, i.e., the nearly total absence of microfilariae during the day. Nor does the maximum number of microfilariae appear at the same time of day in the two major regions. The peak in Tahiti appears to be reached around 6 or 7 p.m. rather than between 10 p.m. and 2 a.m. as is the case in other parts of the world.

A study of the data, particularly in Tables 2 and 5, indicates that examination of a single 20-cmm. blood sample is insufficient to detect all positive persons. The inadequacies of a single 20-cmm. smear in routine surveys have been pointed out by Knott (1939) and others. Beye (unpubl.), however, in making a com-

parative study on a series of 42 subjects, in which he compared the efficiency of the technique used in this study with that recommended by Knott, failed to find the latter technique superior. If one should use the 20-cmm. sample in the South Pacific Area, it is suggested that it would be better to make smears in the afternoon than in the morning. The authors were cognizant of the shortcomings of making a single 20-cmm. smear but considered it satisfactory for this study and for making routine surveys, especially when lack of technical help was a factor. It is thought that the lack of consistent rhythmic fluctuation of the numbers of microfilariae seen in some cases would have been eliminated had counts been based on an average of three or more smears taken at each period of examination. This was clearly demonstrated on a series of three or more smears taken morning and evening for three days from one subject.

It is worthy of note that the persons whose microfilarial counts are shown in Figure 2 were all under 14 years of age. Although only 6 children were studied for two or more days, the periodic fluctuation of microfilariae seemed to be more striking among them than among adults.

SUMMARY AND CONCLUSIONS

Of 26 subjects from one community examined at different hours of the day, on one, two or three days, 24 exhibited greater mean numbers of microfilariae in late afternoon or evening than in the morning. The difference between morning and afternoon levels was highly significant. Maximum numbers of microfilariae appeared in the afternoon with a possible peak around 7 p.m., but there was no evidence of a definite period when minimum numbers were present. Of 2 persons examined at different hours on two days and of 5 persons examined on three days, all except one exhibited a fairly consistent microfilarial fluctuation at each examination.

A study of the microfilarial fluctuation among 13 to 26 persons in each of four widely separated communities revealed that the periodic tendency was common around the island.

Children, aged 6 to 14 years, whose bloods were examined both morning and evening, 4 on six days during the year and one on four days, with one exception, revealed more microfilariae in evening than morning smears.

Microfilarial counts of 10 positive subjects examined once a month during one year and of 6 subjects examined 11 times during one year revealed that December to January was the peak period for microfilariae and April was the low month.

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FIELD STUDIES ON THE RESTING HABITS OF FLIES IN RELATION TO CHEMICAL CONTROL

Part I—In Urban Areas

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Since the discovery of the insecticidal effectiveness of DDT, cities and towns in the United States and throughout the world, in steadily increasing numbers, have instituted municipal fly control programs, using residual or space sprays, or both, as their principal control measure. In these programs, space sprays have been applied during the day aimed at distributing the insecticide over the entire areas where flies were known or thought to be active. Residual sprays were applied on all surfaces considered to be potential fly resting places. In both types of operations, large quantities of materials were used over extensive areas. To effect possible economies and improve the effectiveness of such operations, detailed studies were made at Pharr, Texas, and on a smaller scale at Savannah, Georgia, on the resting habits of the more common flies in urban areas. It was hoped that the information obtained would make it possible to improve the efficiency of chemical control measures by providing a basis for selective treatment with reference both to the timing of space spray applications and to the placement of residuals.

Procedure.—These studies of fly resting habits were begun in the vicinity of Pharr, Texas, in January 1950 and continued through June 1951. The towns selected for these observations were those in which no previous town-wide treatments with residual type sprays had been made or in which the residues of such treatments were of sufficient age as to be of minimum importance. Observations were made on a complete city block basis to include the major shifting of fly populations from daytime feeding places to nighttime resting places. Afternoon and night fly counts were made on the same day in the selected blocks, and counts were made regularly at about weekly intervals throughout the 18 months covered by these investigations. Spotlights and highpowered flashlights were used for illumination in the nighttime observations.

A detailed survey was made of each block and the number and species of flies were recorded for each type of resting place. A differentiation was made between outbuildings and utility buildings. Outbuildings included sheds, garages, animal shelters, etc., which are common to the country as a whole. Utility buildings consisted of small unscreened buildings used for cooking, washing, or temporary shelter for migrant laborers and were more or less characteristic of the area under study. The use of open fires on earthen floors was a common practice in utility buildings. Observations were not made inside of occupied houses during these studies, but casual observations over a period of years in this area have shown that the number of flies found indoors is a relatively small percentage of the total fly population. Most of the houses were screened to some degree.

Musca domestica was the dominant species involved in the studies in the Pharr area, followed in the order of numerical importance by *Sarcophagula* spp. (mostly *occidua*), *Drosophila* spp. (mostly *repleta* and *melanogaster*), *Phaenicia* spp. (mostly *pallescens* and *sericata*), and other miscellaneous species, including *Ophyra leucostoma*, *Ophyra aenescens*, *Callitroga macellaria*, *Sarcophaga* spp., *Fannia pusio*, and a variety of species of several other genera which were relatively rare.

In Savannah, Georgia, similar observations were made in a series of six city blocks during the period July 26 to August 15, 1950. The procedure here varied from that followed in the Pharr area in that the observations in each selected block began before daylight and continued until after dark, with counts being made in only one block during each such observation period. A complete coverage of the block was made before dawn, during each approximate 3-hour period during the day, and again after dark. *Phaenicia* spp (mostly *pallescens*) were dominant in these study blocks, with *M. domestica* second in numbers and *Sarcophaga* spp. a low third. Only occasional specimens of other species were observed, although in other locations, such as around slaughterhouses and garbage dumps, *C. macellaria* were very numerous. Large numbers of *Drosophila* spp. were observed in the Pharr area, but they were rarely seen at Savannah. Most of the houses in the blocks observed were screened. The few flies found inside of the houses were all *M. domestica*.

Results.—The results of the observations in the Pharr area are summarized in table 1. It will be noted from these data that during the day most of the flies, except *Drosophila* spp., were observed on ground surfaces or garbage in containers. "Ground surfaces" included scattered garbage and the soil where dishwater, wash water, table scraps and other materials attractive to flies had been thrown. Approximately three-fourths of the observed house flies, *Phaenicia* spp., and *Sarcophagula* spp., were on these ground surfaces which would not be included in residual spraying operations. About one fifth of *Sarcophagula* spp. and "other" species were found on grasses and weeds and in trees and shrubs during the day, while only small numbers of house flies and *Phaenicia* spp. were observed in these locations. A total of 88 per cent of the observed *Drosophila* spp. was found in privies during the day, nearly all of them resting in the privy pits. From 3 to 20 per cent of the flies other than *Drosophila* were also found in privy pits during the day.

The nocturnal resting places of all species were found to be more suitable for selective application of residual sprays. Tree limbs and shrubs served as the principal nighttime resting places for house flies, and the second most important surface for "other" species, while *Phaenicia* and *Sarcophagula* rested almost exclusively in grasses and weeds. Grasses and weeds served as the second, and wires or lines in utility buildings as the third, most important resting place for house flies, while the ceilings of utility buildings represented the principal resting surface for "other" species. Privy ceilings were the preferred nighttime resting surface of *Drosophila* spp. that were seen, with the privy pits second in importance. However, experience with trapping *Drosophila* in the area showed that large numbers entered homes at night.

Seasonal changes in temperature apparently had little effect upon the resting habits of *Phaenicia* and *Sarcophagula*. However, greater numbers of house flies

TABLE 1

Per cent of common flies found resting during the day and night on the indicated surfaces in the vicinity of Pharr, Texas, from January 1950 to June 1951

SURFACE	PER CENT OF TOTAL FLIES OBSERVED									
	Musca domestica		*Sarcophagula* spp.		*Drosophila* spp.		*Phaenicia* spp.		Other spp.	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
Total Flies Observed	68,466	83,005	15,177	39,102	15,432	30,136	7,018	7,878	4,735	3,437
House:										
Porch walls	†	†	0.0	0.0	0.0	†	0.0	†	0.1	0.0
Porch ceilings	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Sides of house	0.1	0.2	0.0	0.0	0.0	0.0	0.0	†	0.0	0.4
Outbuilding:										
Inside walls	0.1	0.1	†	0.0	1.2	2.6	0.1	0.0	0.2	0.4
Inside ceilings	0.1	0.8	0.0	0.0	0.4	3.8	0.0	0.0	0.1	0.5
Outside walls	0.1	0.8	0.0	0.0	0.0	0.0	0.0	†	0.1	0.1
Outside eaves	0.0	†	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Utility Building:										
Inside walls	0.1	2.7	0.0	0.0	0.1	0.0	0.0	0.0	0.0	8.1
Inside ceilings	†	0.2	0.0	0.0	0.3	4.1	0.0	0.0	0.0	34.4
Wires or lines	†	15.6	0.0	0.0	0.1	3.5	0.0	0.0	†	0.3
Furniture or food	0.7	0.2	0.0	0.0	0.0	†	†	0.0	0.0	0.0
Inside floors	3.0	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
Privy:										
Walls	0.0	0.0	0.0	0.0	2.2	1.9	0.0	0.0	0.0	0.0
Ceilings	0.0	2.5	0.0	0.0	0.1	62.1	0.0	0.0	†	1.0
Pits	3.4	0.5	9.2	0.4	85.7	17.9	3.6	0.5	19.9	4.6
Seat or floor	0.6	0.0	0.1	0.0	0.1	†	†	0.0	0.2	0.2
Vegetation:										
Tree limbs—shrubs*	2.6	43.6	1.7	0.4	0.0	1.6	1.4	3.8	18.0	28.9
Grasses—weeds	2.1	25.7	17.3	98.8	0.0	0.2	4.4	94.6	4.2	17.8
Tree trunks	†	0.2	0.0	†	0.0	0.0	0.0	†	0.0	0.2
Fence:										
Wires or slats	6.6	4.1	1.3	0.4	0.0	0.8	2.2	0.8	12.4	1.8
Posts	†	†	0.0	0.0	0.0	0.0	†	0.0	0.1	0.0
Other:										
Bottles, boxes, cans, etc.	0.6	0.0	0.4	0.0	0.0	0.3	1.4	0.0	0.1	0.0
Furniture—outside	0.2	0.0	0.0	0.0	0.0	†	0.0	0.1	†	†
Ground surfaces	66.2	†	68.4	0.0	1.7	0.0	74.9	†	30.5	0.0
Garbage in containers	10.2	0.3	1.1	0.0	7.0	1.2	11.6	0.1	3.7	0.2
Clothes lines	2.6	2.0	0.3	0.0	†	†	0.1	0.1	9.6	0.6

* Includes foliage.

† Less than 0.05 per cent.

were found at night in the utility buildings and on privy ceilings during the January–March quarters of 1950 and 1951 than during any other period. These flies apparently moved from exposed to more protected resting surfaces as daily

TABLE 2

Fly observations in six city blocks of Savannah, Georgia, at indicated times during the day showing resting surfaces with number and per cent of flies per resting place by species, July 26–August 15, 1950

RESTING PLACE	SPECIES	PREDAWN	5:30 TO 8:30 A.M.	8:30 TO 10:30 A.M.	10:30 TO 1:30 P.M.	1:30 TO 4:30 P.M.	4:30 TO 7:30 P.M.	AFTER DARK	AV. % OF TOTAL FLIES	
									Night	Day
Grasses and weeds	Pha.*	26071	11474	3140	4832	3147	3041	25701	99.4	27.9
	M.d.*	1218	106	47	54	80	80	780	48.9	4.9
	Sar.*	3	5	2	3	1	1	6	45.0	10.6
Clothes lines	Pha.	32	18	17	31	48	3	44	0.1	0.1
	M.d.	203	139	65	97	82	23	309	12.5	5.4
	Sar.	0	1	0	0	1	0	0	0.0	1.8
Trees and shrubs	Pha.	97	15	9	29	15	0	114	0.4	0.1
	M.d.	481	91	42	55	50	6	759	30.4	3.2
	Sar.	3	0	0	4	2	0	7	50.0	5.3
Fences	Pha.	9	66	15	6	8	1	9	0.0	0.1
	M.d.	24	89	3	14	16	5	31	1.3	1.7
	Sar.	1	2	0	0	0	0	0	5.0	1.8
Porches and steps	Pha.	0	0	113	60	39	22	1	0.0	0.3
	M.d.	20	86	212	166	143	33	10	0.7	8.5
	Sar.	0	0	0	0	0	0	0	0.0	0.0
Ground	Pha.	0	7231	7360	8565	10317	8650	0	0.0	45.8
	M.d.	0	1126	1112	981	835	207	0	0.0	56.8
	Sar.	0	9	12	14	17	7	0	0.0	52.2
Garbage	Pha.	0	1092	8063	6043	4784	3295	0	0.0	25.3
	M.d.	0	181	254	125	116	74	0	0.0	10.0
	Sar.	0	9	6	2	6	3	0	0.0	23.0
Sides of bldgs.	Pha.	0	251	46	15	12	11	1	0.0	0.4
	M.d.	0	153	36	27	22	7	0	0.0	3.3
	Sar.	0	6	0	0	0	0	0	0.0	5.3
Inside house	Pha.	—	0	—	0	—	—	0	0.0	0.0
	M.d.	—	225	—	242	—	—	248	6.1	6.2
	Sar.	—	0	—	0	—	—	0	0.0	0.0
Totals	Pha.	26209	20147	18763	19581	18370	15023	25870	99.9	100.0
	M.d.	1946	2196	1771	1761	1344	435	2137	99.9	100.0
	Sar.	7	32	20	23	27	11	13	100.0	100.0

* Pha.—*Phaenicia* spp., mostly *pallescens*
M.d.—*Musca domestica*
Sar.—*Sarcophaga* spp.

minimum temperatures dropped. *Drosophila* spp. appeared to be more dispersed at night from July to December; however, this was the period when *Drosophila* were at their seasonal low point in numbers and the observed flies were only a small percentage of those noted from January to June. During the first three months of 1951, a period of unusually low temperatures, a high percentage of *Drosophila* was found on garbage in containers during both day and night, although the total number observed was small.

The results of the observations in Savannah, Georgia, are shown in table 2. The resting habits of the house flies and of *Phaenicia* were found to be almost identical with those observed at Pharr, Texas, during the same period. Ground surfaces and scattered garbage served as the principal resting surfaces for both species during the day, although 28 per cent of the *Phaenicia* in Savannah were found on grasses and weeds as compared to only about 5 per cent in the Pharr area. From July 26 to August 15, 1950, approximately 79 per cent of the house flies were found in grasses and weeds or trees and shrubs at night in Savannah, as compared to 96 per cent at Pharr. *Phaenicia* were observed on grasses and weeds almost exclusively in both areas. No detailed observations were made on *C. macellaria* during these studies, but casual observations of the resting habits of this species made over a period of years in connection with other investigations have shown that they rest at night almost exclusively on weeds, grasses, trees, and shrubs.

Discussion.—Based on the observations of fly resting habits in the vicinity of Pharr, Texas, and in Savannah, Georgia, it would appear that residual spray operations for fly control in urban areas should include the treatment of grasses and weeds, the lower limbs of trees, shrubs, garbage containers and their immediate environs, and the insides of privies, including their pits. In the Pharr area, consideration also should be given to treating the inside of utility buildings.

The selective spraying of such places would result in the treatment of the principal nocturnal and some of the daytime resting surfaces, of most of the flies commonly found in urban areas. Thus, the majority of flies would be brought into contact with the spray residues for the longest possible time. The general spraying of outbuildings and the outside of houses or other structures was not indicated by these studies. The elimination of the treatment of such surfaces would provide ample materials to treat the grasses, weeds, shrubs or bushes, and the lower limbs of trees, and still effect savings in the cost of operations.

The high concentration of flies at night in weeds, grasses, shrubs, and trees suggests the possibility that more efficient space spraying operations might be accomplished in many urban areas by selective nighttime treatment of these resting places.

Of particular interest in the Pharr studies was the observed preference of *Drosophila* spp. (*repleta* and *melanogaster*) for privies. During the day most *Drosophila* were found in privy pits, whereas at night most of them were observed on privy ceilings. This would indicate that *Drosophila* may be more active at night than during the day. The specimens noted in the privy pits during the day were observed in about equal numbers on the sides of the pits and on the

contents in the bottom of the pits. Whether the flies were attracted to the privy pits for feeding, oviposition and/or resting was not determined. The presence of *Drosophila* in great numbers in privies raises the question of their possible role as filth flies and disease vectors. These flies are sufficiently small to be unnoticed in situations where larger flies would attract attention, and some species readily pass through screening ordinarily used in homes. Furthermore, they are attracted mainly to those types of food which are eaten raw. These facts indicate a definite need for further studies on *Drosophila* to determine their status in relation to the transmission of human disease.

Summary.—Diurnal and nocturnal observations of fly resting habits in urban areas were made in the vicinity of Pharr, Texas, from January 1950 to June 1951 and in Savannah, Georgia, during a short period in the summer of 1950.

M. domestica was the most numerous species observed at Pharr, followed in order of numerical importance by *Sarcophagula occidua*, *Drosophila* spp. (*repleta* and *melanogaster*), and *Phaenicia* spp. (mostly *pallescens* and *sericata*). In Savannah *Phaenicia pallescens* was by far the most numerous species observed, with *M. domestica* second and *Sarcophaga* sp. third.

In both areas, ground surfaces and scattered garbage were the principal daytime resting surfaces for all observed species except *Drosophila*. Likewise, grasses, weeds, trees, and shrubs represented the most important nocturnal resting surfaces for all species except *Drosophila*.

The observations on *Drosophila* were confined entirely to the Pharr area. During the day, most of the *Drosophila* were found in privy pits, whereas at night most of them were observed on privy ceilings.

Based on these studies, residual spray operations for urban fly control should include the treatment of grasses, weeds, shrubs, the lower limbs of trees, the interior of privies, and garbage cans and their immediate environs. In the Pharr area, the interior of utility buildings should also be included. The spraying of outbuildings or the outsides of any structures was not indicated.

The high concentration of flies in preferred resting places at night suggests that selective space spraying of these resting places at night might give more efficient fly control than present daytime space spraying procedures.

The presence of great numbers of *Drosophila* in privies raises the question of their possible role as filth flies and disease vectors and emphasizes the need for further studies to determine their status in relation to the transmission of disease.

REFERENCE

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FIELD STUDIES ON THE RESTING HABITS OF FLIES IN RELATION TO CHEMICAL CONTROL

Part II—In Rural Areas

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During the past several years increasing difficulty has been encountered in the control of house flies by chemical means, due to their development of resistance to a variety of insecticides. Consequently, it has become necessary to delve into the more basic aspects of fly activities and behavior in order to develop new approaches to control. Observations of the diurnal and nocturnal resting habits of flies were carried on from the spring of 1950 to the summer of 1951.

Procedure.—These studies of fly resting habits were made in rural districts near Savannah, Georgia. The farm units are located from one-quarter to several miles apart and many are separated by extensive woodlands. The premises selected for the observations of fly resting habits varied from small, isolated shacks to large dwellings with surrounding outbuildings housing livestock, poultry and equipment. Both screened and unscreened dwellings were included.

Counts of the total fly population were made on each premise, and detailed records were made of the type of surface on which the flies were found to be resting. Undoubtedly some flies were not counted, due to the fact that all potential resting surfaces were not readily observable. However, the consistency of the data on the same premises at different times during the same day indicates that a relatively accurate population count was obtained in most instances.

Results.—Musca domestica was the dominant species observed in these rural districts, representing such a high percentage (97 per cent) that no species breakdown was considered necessary in presenting the tabular data which appear herein. *M. domestica* was the only species observed inside houses, except for a few *Drosophila* spp., and very rarely *Sarcophaga* spp. or *Phaenicia* spp.

Table 1 shows the general trend of the resting habits of flies on 68 rural premises, both screened and unscreened, between the hours of 8:30 a. m. and 4:00 p. m. under a variety of spring and early summer temperature and weather conditions. It will be noted from this table that in the screened dwellings less than one per cent of the total number of flies observed were found inside the house as compared with about 20 per cent inside unscreened dwellings. This would indicate that screens were more than 97 per cent effective in preventing flies from gaining entrance into homes. A large percentage of the flies found inside dwellings were in the kitchen, a health hazard which could be greatly reduced with the use of proper screening.

An item of utmost importance from the standpoint of residual spraying operations was the small percentage of flies observed resting on surfaces which are normally sprayed. In the present studies, only about 12 per cent of the flies observed on screened premises were resting on normally sprayed surfaces (Table

1), and 21 per cent on unscreened premises. The higher percentage on unscreened premises was due primarily to the larger number of flies resting on walls and ceilings inside the house.

TABLE 1

Daytime observations of flies resting in various locations on rural premises with screened and unscreened dwellings, showing per cent of total flies for each location along with per cent resting on a sprayable surface, April 26 to July 20, 1950*

LOCATION ON PREMISES	SCREENED DWELLING			UNSCREENED DWELLING		
	No. of flies observed on 20 premises	Per cent of total flies	Per cent of total flies resting on sprayable surface	No. of flies observed on 48 premises	Per cent of total flies	Per cent of total flies resting on sprayable surface
Kitchens	228	0.29	0.04	3,136	7.22	1.69
Bedrooms	116	0.15	0.01	2,895	6.66	0.41
Living rooms	75	0.10	0.01	2,068	4.76	0.41
Dining rooms	13	0.02	0.00	612	1.41	0.12
Front porch & sides of house	338	0.43	0.27	1,488	3.43	2.98
Back porch	486	0.62	0.46	932	2.15	0.53
Animal pen floors	57,022	73.02	0.00	7,854	18.08	0.00
Animal pen fences	1,168	1.50	1.50	930	2.14	2.14
Other fences	389	0.49	0.49	171	0.39	0.39
Ground & dishwater	1,916	2.45	0.00	3,014	6.94	0.00
Grass, weeds & misc.	4,451	5.70	4.22	4,469	10.30	5.86
Trees & shrubs	824	1.06	1.06	468	1.07	1.07
Chicken house						
Inside	354	0.46	0.26	253	0.58	0.41
Outside	140	0.18	0.18	98	0.23	0.23
Barns						
Inside						
Floors	7,850	10.06	0.00	12,494	28.77	0.00
Other surfaces	1,550	1.98	1.62	865	1.99	1.01
Outside	505	0.65	0.65	530	1.22	1.22
Privy						
Inside	361	0.46	0.46	876	2.02	2.02
Outside	90	0.12	0.12	131	0.30	0.30
Misc. outbldgs.						
Inside	103	0.13	0.13	100	0.23	0.12
Outside	116	0.15	0.15	50	0.12	0.12
Totals	78,095	100.02	11.65	43,434	100.01	21.03

* 97 per cent *M. domestica*

Observations were made on another group of 15 rural premises having unscreened dwellings, starting before daylight in the morning and continuing throughout the day until after dark. Only one premise was observed each day. These observations (Table 2) revealed resting habits which lend themselves better to chemical control. In the early predawn hours and at night, 88 per cent of the total observed flies were found to be resting inside the unscreened house;

another 7 per cent were found resting in trees and shrubs. At no other time during the 24-hour period of each day could 95 per cent of the fly population be found resting in such a limited area for such an extended period of time. Most of the flies resting inside the house were on the ceilings, with the remaining few on the upper portion of the walls, usually within 3 or 4 feet of the ceiling. During the main daylight hours (7:30 a.m. to 5:30 p.m.) there was a greater dispersal of flies over the entire premises, and most of the flies found inside the dwellings were on tables, floors, cabinets, furniture, and other surfaces that are not usually sprayed. The most intensive period of activity was from approximately 9:00 a.m.

TABLE 2

House fly observations on 15 rural premises with unscreened dwellings at indicated times during the day showing resting surfaces, number of flies per resting place, and average per cent of total flies, June 13 to October 25, 1950

RESTING PLACE	NIGHT	DAY						NIGHT	AV. % OF TOTAL FLIES PER RESTING PLACE	
	Pre-dawn	5:30–7:30 A.M.	7:30–9:30 A.M.	9:30–11:30 A.M.	11:30–1:30 P.M.	1:30–3:30 P.M.	3:30–5:30 P.M.	After dark	Night	Day
Ground	0	131	1186	2188	1804	1065	949	0	0.0	10.1
Porch	301	622	602	482	491	603	634	309	2.6	4.8
Trees and shrubs	887	544	218	307	312	268	352	785	7.2	2.7
Barn walls inside	148	125	153	143	213	199	144	155	1.3	1.4
Barn walls outside	2	83	203	137	125	114	69	6	0.0	1.0
Barn floors	0	130	267	693	1067	1508	679	29	0.1	6.0
Floors of animal pens	0	37	127	473	722	505	361	0	0.0	3.1
Sides of misc. bldgs.	8	23	155	91	119	92	50	14	0.1	1.0
Sides of house	7	136	161	129	152	122	235	18	0.1	1.3
Dishwater and garbage	0	162	441	671	896	873	686	0	0.0	5.2
Clothesline	14	14	2	5	13	9	5	21	0.2	0.1
Fences	55	157	149	125	109	98	115	68	0.5	1.0
Inside house	9955	9679	8137	6343	6076	6877	8064	10532	87.9	62.6
Totals	11377	11843	11801	11787	12099	12333	12343	11937	100.0	100.3

to 4:00 p.m. Counts during this period were highest on the ground, dishwater, garbage, and in animal pens and stalls.

Flies were found resting in small numbers at night on the porches of unscreened dwellings, with an increase noted in the counts soon after sunrise and again just before dark. These increases tend to verify the movement of flies from the dwellings in the morning and back into them at night.

Early in the studies, observations were made throughout the night to determine if any fly movement occurred after darkness. Observations were made on both dark and brightly moonlit nights. Flies began seeking out night resting places between sundown and dark. Shortly after darkness had fallen, all flies were settled for the night and remained in essentially the same places until early daylight or sometime after sunrise, remaining later on cool or cloudy days. Light or moderate rainfall did not alter the night resting habits.

During the late summer and fall of 1950 temperatures appeared to influence greatly the nocturnal resting habits of the flies on screened premises. As the prevailing temperatures dropped, the flies shifted their resting places from trees and shrubs to more protected locations such as barns and porches. During the height of winter, when freezing temperatures occurred periodically, a few adult flies could be found at all times inside unscreened premises. Adult flies on screened premises disappeared after each period of freezing weather, and reappeared only after several days of continuous warm weather.

TABLE 3

Summary of nighttime observations during the period March 1 to August 24, 1951, on the resting habits of house flies on three rural unscreened premises

RESTING SURFACE	NUMBER OF FLIES OBSERVED				
Trees	173	30	438	216	56
Barns	172	12	150	224	71
Porches	12	56	409	91	66
Inside house	3,197	1,679	11,387	2,816	1,848
Other	3	16	47	2	6
Minimum temperature range in °F	49–50	51–52	56–60	64–65	69–74
Maximum temperature range in °F	69–81	81	70–90	89–93	87–97
No. replications	3	2	8	2	2

TABLE 4

Nighttime observations during the period March 22 to August 23, 1951, on the resting habits of house flies on a typical rural screened premise

RESTING SURFACE	NUMBER OF FLIES OBSERVED			
Trees	7	0	1,391	3,749
Barns	171	74	431	167
Porches (unscreened)	211	36	4,238	814
Other	18	0	19	16
Minimum temperature range in °F	41–42	53	58–60	69–72
Maximum temperature range in °F	63–73	74	80–89	88–95
No. replications	3	1	3	3

To follow more carefully the effects of temperatures on the nocturnal resting habits of the flies, observations on three unscreened premises were begun in the early spring of 1951 and continued into the summer. Minimum temperatures ranged from 49–74°F. and maximum temperatures ranged from 69–97°F. (Table 3). Results of observations on the three premises show essentially the same picture regardless of temperatures. An average of approximately 90 per cent of the flies rested inside the house at night, with most of these resting on the ceilings.

Similar nighttime observations were made on three screened premises with minimum and maximum temperatures ranging from 41–72°F. and 63–95°F.,

respectively. The results of the observations on one of these premises, which was considered a typical farm unit with the doors and windows of the house screened but the porches unscreened, are shown in Table 4. At this place approximately 95 per cent of the fly population at the lower temperatures (41–53°F. minimum) were found either inside the barn or on the porches. As temperatures rose (58–60°F. minimum) the flies began to move into the trees. With minimum temperatures in the neighborhood of 70°F., approximately 80 per cent were found in the trees, with most of the remainder on the porches.

The observations on the other two screened premises, on which the back porches were also screened, showed similar trends in the resting habits of the flies.

Discussion.—One of the more surprising features of these studies was the absence of flies resting in barns at night, especially during the main fly breeding season when higher temperatures prevailed. They were found resting inside barns at night only when they were apparently forced to do so by low temperatures. This failure of the flies to use the barns extensively as night resting places during most of the active fly season tends to reduce the value of residual spraying of barns for fly control. However, since some flies were observed resting in the barns at all times and large numbers were observed on barn floors during the day, it is likely that residual treatment of the barn interiors would bring a considerable percentage of the flies into contact with the insecticide during the day. Furthermore, since barns are frequently the only or major source of fly breeding on the premises, treatment of the barn walls would expose most newly emerged flies to the insecticidal residues immediately after emergence. Very early and again late in the season, practically the entire fly population on screened premises would be exposed to barn treatments while resting at night.

Trees and shrubs serve as important fly resting places, especially at night during the warm part of the year. On trees the flies were found, for the most part, on the smaller twigs and on the under sides of the leaves. In the larger trees, practically all of the observed flies were found on the lower branches within 10–15 feet above the ground. In trees near dwellings, the flies tended to congregate on the side nearer the house, especially near kitchens.

In general, these observations have shown that house flies in rural areas prefer to rest on surfaces that are protected from the weather and on surfaces that can be easily grasped with the feet. Wires or cords on back porches or in other protected places such as open work houses proved to be desirable resting places, whereas clotheslines and wire fences without a protective covering were used much less frequently. Similarly, small dead twigs and limbs protected by a canopy of leaves were favored resting surfaces, while twigs of similar size and shape without an overhead covering of leaves were seldom used.

Residual spray applications for fly control on rural premises should include unscreened porches, the lower portions of trees and shrubs adjacent to the dwelling or to animal shelters, and that portion of the barn interiors which animals use. The ceilings and upper three feet of the interior walls of unscreened dwellings should be sprayed thoroughly. It was not indicated on any type of premises

that the exteriors of dwellings or outbuildings should be sprayed. From the standpoint of fly control alone, spraying inside screened dwellings does not appear indicated. Because of the high efficiency of screening in excluding flies from homes, consideration might well be given, in planning long-range fly control operations in rural areas, to the relative costs of screening as compared to the repeated spraying of the homes.

The high concentrations of flies at night in relatively small areas suggest the possibility of controlling flies on rural premises by the selective application of space sprays at night. In many instances this could be accomplished with conventional compressed-air hand sprayers equipped with an atomizing nozzle and an extension wand. Because of the relatively small quantities of material that would be required, more expensive materials could be used. This method might be especially useful against resistant strains of flies.

SUMMARY

Musca domestica was the dominant species (97 per cent) observed in studies of fly resting habits in rural areas near Savannah, Georgia, 1950–1951. During the daytime hours only small percentages of the flies were observed resting on surfaces which are normally sprayed. In unscreened houses the greatest concentrations of flies during the day were found in the kitchens. Repeated all-night observations showed that flies remained in one place during the entire night.

Screens were indicated to be more than 97 per cent effective in preventing flies from gaining entrance into homes. On unscreened premises approximately 90 per cent of the flies were found resting in the houses at night throughout the year. On screened premises in cooler temperatures flies were found resting at night in barns; with slightly warmer temperatures they began moving out to porches, and as minimum temperatures approached 70°F., most of the flies were found in the lower branches of trees and on shrubs.

Residual spray applications for fly control on rural premises should include the treatment of porches, the lower portions of trees and shrubs adjacent to houses and animal shelters, barn interiors, and the ceilings and upper portions of the walls of unscreened dwellings. The high concentrations of flies at night in relatively small areas suggest the possibility of controlling flies economically by the selective application of space sprays at night.

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FIELD STUDIES ON THE USE OF INSECTICIDES TO CONTROL FLY BREEDING IN GARBAGE CANS

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Surveys by Quarterman *et al.* (1949) established the fact that the garbage can is the principal source of fly production in the residential districts of Savannah, Georgia. This has been substantiated by the studies of Maier and Baker (1951) at Pharr, Texas, an area that is widely different in climatic conditions and population make-up. It is recognized that the most desirable solution to this problem is an enlightened public that will prevent fly breeding in garbage cans by keeping the cans clean. This, however, can be accomplished only after the establishment of an adequate educational program supplemented by the enactment and enforcement of suitable local sanitation ordinances. In the meantime some other means of controlling fly breeding in garbage cans is needed for use in operational fly control programs in many urban areas. The tests discussed herein were designed to provide data on the possible use of certain chemicals for this purpose.

PROCEDURE

During 1950 a series of fly infested garbage cans was selected for treatment with candidate chemicals in Savannah, Georgia. Only cans with active larval infestations in the sludge in the bottom of them were selected for treatment. The infested cans were treated inside and outside with the candidate chemical applied as a water emulsion. The treated cans together with untreated check cans were brought immediately to the laboratory grounds where lids equipped with a small emergence trap were sealed on them. Emerging flies were removed from the emergence cages to holding cages, offered food and water and held in the laboratory for 24-hour mortality checks.

After being held for approximately one month for fly emergence, the cans were returned to their owners and again placed in normal use without being washed or cleaned out. After approximately one month in normal use, the cans were collected a second time and held at the laboratory for further emergence checks.

During 1951 additional tests were conducted on a more limited scale using a modified procedure for checking on the duration of effectiveness of the treatments. Technical BHC was applied to both the inside and outside of infested cans as a 5-per cent emulsion at the rate of 200 mg. of BHC per square foot. PDB (paradichlorobenzene) was applied by distributing the crystals evenly over the bottom sludge in the cans at the rate of 2 ounces of PDB per can.

Forty-four cans were treated with each material in 1951. Approximately 15 cans treated with each material were brought to the laboratory at the following intervals: group 1, immediately after treatment; group 2, 1 week after treatment; group 3, 2 weeks after treatment. Groups 2 and 3 were in normal use after treatment until they were brought to the laboratory. The cans were held at the laboratory only one week for emergence checks.

RESULTS

The results of the 1950 treatments in controlling the fly breeding present in the cans at the time of application are shown in table 1. It is apparent that

TABLE 1

Number of adult flies emerging from garbage cans during the first month after treatment with the indicated chemicals. The cans were taken out of normal use during this period of observation. Savannah, Georgia, 1950

INSECTICIDE	CHECK		DIEL-DRIN		DIELDRIN		DIELDRIN + ROSIN		CHLORDAN		CHLORDAN		LINDANE		BHC	
Dosage—mg./sq. ft.	0		25		50		50		100		200		50		200	
No. of Cans	9		16		25		16		16		16		34		13	
Fly Species	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Musca domestica	16	249	10	38	20	102	23	91	8	181	220	1406	14	96	0	0
Phaenicia pallescens	0	6	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Callitroga macellaria	0	270	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hermetia illuscens	1	34	2	3	7	8	31	19	9	49	9	20	0	2	0	14
Fannia pusio	3	10	1	0	30	0	1	0	13	0	66	2	0	0	1	0
Sarcophaga bullata	0	1	0	0	0	0	0	0	0	0	4	1	0	0	0	0
Dendrophaonia hilariformis	15	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piophila casei	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychodidae	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous species*	0	4	0	0	3	0	0	0	0	0	1	1	0	0	0	0
Totals—all species	37	624	13	41	60	110	56	110	30	230	302	1430	14	98	1	14
Avg. per can—all species	73		3		7		10		16		108		3		1	
Range of total emergence per can	0–253		0–39		0–61		0–78		0–77		0–410		0–25		0–6	
Number of cans from which some flies emerged	8		7		14		9		15		14		15		5	
Number of cans from which no flies emerged	1		9		11		7		1		2		19		8	

D—Dead on removal from emergence cage or died within 24 hours.

S—Survived more than 24 hours after emergence.

* Miscellaneous species include 1 *Sarcophaga sarracenioides*, 2 *Muscina stabulans* and 1 *Euzesta notata* survived in the group of checks. Two *M. stabulans* and 1 *Stomoxys calcitrans* dead from cans treated with 50 mg./sq. ft. of dieldrin; and 1 *Drosophila robusta* dead and 1 *Euzesta apicalis* survived from the cans treated with 200 mg./sq. ft. of chlordan.

dieldrin, lindane and BHC only partially suppressed the fly production in the treated cans, with BHC giving the best results. Chlordan was relatively ineffective against house flies and a common soldier fly, *Hermetia illuscens*, but seemed to be effective against most of the other species breeding in the cans including the more common blow flies.

The house fly, *Musca domestica*, was the dominant species breeding in the cans. Most of the *Callitroga macellaria* shown in table 1 for the check cans emerged from one can, although some emergence of this species occurred in 3 of the 9 check cans.

TABLE 2

Number of adult flies emerging from garbage cans during the third month after treatment with the indicated insectides. The cans were in normal use during the second month, after which they were removed for emergence checks. Savannah, Georgia, 1950

INSECTICIDE	CHECK		DIELDRIN		DIELDRIN		DIELDRIN & ROSIN		LINDANE		BHC	
Dosage—mg./sq. ft.	0		25		50		50		50		200	
No. of cans	9		15		24		13		32		13	
Fly species	D	S	D	S	D	S	D	S	D	S	D	S
Musca domestica	1	22	17	177	11	153	184	1737	21	513	1	59
Fannia pusio	7	20	12	3	0	3	0	0	0	60	0	0
Muscina assimilis	0	0	0	0	0	2	0	0	0	9	0	0
Misc. species*	1	4	1	0	3	4	0	0	0	3	2	1
Totals—All species	9	46	30	180	14	162	184	1737	21	585	3	60
Avg. per can—all species	6		14		7		148		19		5	
Range of total emergence per can	0–40		0–65		0–40		0–611		0–197		0–26	
No. cans from which some flies emerged	5		6		10		11		11		7	
No. cans from which no flies emerged	4		9		14		2		21		6	

D—Dead on removal from emergence cage or died within 24 hours.

S—Survived more than 24 hours after emergence.

* Miscellaneous species include 1 *Drosophila repleta* dead plus 2 *Hermetia illuscens* and 2 *Muscina stabulans* survived in check cans; 1 *Psychodidae* dead in cans treated with 25 mg./sq. ft. of dieldrin; 1 *Phaenicia pallescens* plus 2 *Fannia canicularis* dead and 2 *P. pallescens* plus 2 *Sarcophaga lambens* survived in cans treated with 50 mg./sq. ft. of dieldrin; 3 *Sarcophaga bullata* survived in cans treated with 50 mg./sq. ft. of lindane; and 1 *F. canicularis* plus 1 *Calliphora livida* dead and 1 *P. pallescens* survived from cans treated with 200 mg./sq. ft. of BHC.

Table 2 shows the fly emergence from the 1950 test cans after they had been held at the laboratory for one month for the fly emergence discussed above, returned to the owners for normal usage for one month, and again brought to the laboratory for emergence checks. The month of usage occurred during the second month after treatment, and the data in table 2 represent the emergence subsequent thereto. None of the treatments was effective in preventing fly infestation during the second month when the cans were in normal use.

The results of the 1951 tests with BHC and PDB are given in table 3. House flies were able to emerge and survive in small numbers from each of the three

groups of cans treated with BHC. The number surviving increased with the time the cans remained in use after treatment and by the end of the second week in use one can produced a total of 39 house flies, of which 25 survived. Only one treated can produced anything other than house flies and these died during the holding period. In the check cans 41 per cent of the flies produced were species other than house flies. The majority of these were *F. pusio*, followed by *Phaenicia pallescens*. The other species involved were in very small numbers.

TABLE 3

Number of adult flies emerging from garbage cans treaged with technical BHC or paradichlorobenzene. Savannah, Georgia, 1951

INSECTICIDE	CHECK		TECHNICAL BHC						PARADICHLOROBENZENE					
Dosage	—		200 mg./sq. ft.						2 oz. per can					
Number of cans	26		14		15		15		15		17		12	
Length of time cans in use after treatment	—		0		1 wk.		2 wks.		0		1 wk.		2 wks.	
Fly species	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Musca domestica	15	211	8	9	1	12	23	62	0	0	0	0	4	14
Fannia pusio group	21	89	5	0	0	0	0	0	0	0	0	0	3	56
Fannia canicularis	0	1	6	0	0	0	0	0	0	0	0	0	0	6
Phaenicia pallescens	0	36	0	0	0	0	0	0	0	0	0	0	0	10
Stomoxys calcitrans	0	6	0	0	0	0	0	0	0	0	0	1	0	0
Total—all species	36	343	19	9	1	12	23	62	0	0	0	1	7	86
Avg. per can—all species	14.8		2.0		1.0		5.7		0		0.6		7.7	
Range of total emergence per can	0–78		0–19		0–7		0–39		0		0–1		0–60	
No. cans from which some flies emerged	16		5		4		7		0		1		4	
No. cans from which no flies emerged	10		9		11		8		15		16		8	

D—Dead on removal from emergence cage or died within 24 hours.

S—Survived more than 24 hours after emergence.

Two *Hermetia illuscens* and 1 each of *Sarcophaga bullata*, *Sarcophaga sarracenioides* and *Hydrotea houghii* survived from check garbage cans in addition to the species listed above.

The failure of the BHC completely to control the breeding of house flies in the cans treated with it in 1951 is considered as further evidence to substantiate observations from other studies that house fly resistance to BHC and other chlorinated hydrocarbons was increasing steadily during 1950–51 in the Savannah area.

In the cans treated with PDB no flies emerged from containers that were brought in immediately after treatment and only 1 specimen (*Stomoxys calcitrans*) emerged from the ones that remained 1 week in normal use after treatment. The group that was brought in 2 weeks after treatment produced a total

of 86 flies that survived, of which 14 were house flies. Of this number, one can produced 60 flies that survived, only 2 being house flies.

DISCUSSION

An interesting feature of these studies, aside from the results obtained with the test insecticides, was the data on fly emergence from garbage cans during the winter months. The last set of cans in 1950 were brought to the laboratory for emergence checks late in October. Emergence of house flies began on November 10, 1950, and continued steadily with a break of no more than 3 days at a time until November 24. Minimum daily temperatures had begun to fall into the thirties during this period and freezing temperatures occurred on November 24. On that date house flies emerged from two cans and *Muscina assimilis* from another, and all survived upon removal to the laboratory. A severe cold wave arrived on November 25 dropping the temperature to a low of 15°F. on that date, with a maximum of 32°F. The minimum temperatures during the next 5 days ranged from 21°F. to 33°F. and the maximum temperatures from 47°F. to 62°F. On November 30, following a low of 30°F. that morning and the prolonged period of cold weather mentioned above, house flies emerged from one can and survived when transferred to the laboratory.

During the balance of the winter, there were frequent periods of cold weather lasting from 3 to 6 days when minimum temperatures fell below freezing daily. House flies continued to emerge throughout this period and there was also some emergence of *F. pusio* and *M. assimilis*. All species survived satisfactorily upon removal to the laboratory. Emergence of house flies continued until March 2. On March 6–8, several specimens of *F. pusio*, *P. pallescens*, and *Sarcophaga bullata* emerged from two of the cans.

The winter of 1950–51 was one of the coldest winters experienced in the vicinity of Savannah in many years. Temperatures were subnormal during a major part of the winter. The garbage cans from which the flies emerged sporadically throughout the winter were stored outdoors in a grove of trees. On the basis of these limited observations it would appear that several of the common species of flies in this vicinity, especially house flies, emerge in small numbers throughout normal winters and that garbage cans containing bottom sludge serve as an overwintering site for them.

SUMMARY

In tests at Savannah, Georgia, during 1950 with technical BHC (12 per cent gamma isomer), lindane, dieldrin and chlordan, best results were obtained with technical BHC for controlling fly breeding present in the bottom sludge of garbage cans at the time of treatment. Chlordan was the least effective. In 1951 tests, PDB crystals were more effective than technical BHC. No material tested either year was effective in controlling fly breeding in garbage cans beyond 2 weeks after treatment. All materials tested appeared to be more effective against the more common blow flies than against house flies.

Several common species of flies, including house flies, emerged sporadically

from garbage cans throughout the winter of 1950–51, during which there were numerous cold spells with minimum temperatures as low as 15°F. Emergence frequently occurred on days when the minimum temperatures were well below freezing, 21°F. being the lowest.

ACKNOWLEDGMENT

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EXPERIMENTAL STUDY ON THE IMPORTANCE OF EARLY ANTIVENIN TREATMENT IN SNAKE BITE[1, 2]

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In snake bite accidents, there is a variable period of time between the moment when the victim receives a possible lethal dose of rapidly spreading venom and the commencement of antivenin treatment, an interval that might greatly influence the outcome of the case. Therefore it is of vital interest to investigate how such a delay affects the curative efficacy of an antivenin dose sufficient to neutralize a fatal amount of venom if administered at once. As severe snake bite accidents are too rare to permit statistical evaluation of the benefits of antivenin treatment, only properly designed laboratory experiments, as also advocated by Allen (1938), may settle the problem in question. Researches in this direction were undertaken by Brazil (1909), Gomes (1920), and Menk (1930), but a complete lack of details regarding, for instance, the identity of the venom examined, the inconstancy of experimental conditions, or the very small number of animals used in the tests make it impossible to draw definite conclusions from the papers of these authors.

MATERIAL AND METHOD

For the purposes of the present study an approximately lethal dose of venom was injected subcutaneously in the left hind dorsal quarter of female mice weighing 15 to 22 gm. Immediately after the venom inoculation, or after 1, 2, 3, 4, 5, or 6 hours, antivenin in a dose of 0.01 ml. per gm. of body weight was injected subcutaneously in the corresponding right side of the animals or into a caudal vein, while one lot of mice remained untreated to serve as a control of toxicity. Twenty animals were used in each test, giving a total of 300 per series. Two types of venom were examined, that of *Bothrops jararaca*, which causes circulatory collapse and extensive local necrosis, and that of *Crotalus terrificus*, which provokes respiratory failure by peripheral paralysis. The venom doses were 0.05 mg/gm. in the case of *Bothrops* and 0.001 mg/gm. for *Crotalus*. The antivenins were 10 per cent solutions of the preservative-free lyophilized antibothropic serum B-114 (genus specific) and anticrotalic serum C-100 (species specific) of the Instituto Butantan. Both of the sera had been concentrated and purified by treatment with ammonium sulphate and pepsin. The 300 single experiments of each series were carried out on the same day with the same solutions of venom and antivenin. Observation time was recorded till death or complete recovery.

RESULTS

In the experiments with *Crotalus* venom, all untreated control animals died in less than 4 days, and 6 of the mice chosen for 5 or 6 hour treatments were dead

[1] This study was carried out under Government of the State of São Paulo (Public Health) Contract Reg. No. TC 3861-50.

[2] Presented at the Staff Meeting of the Instituto Butantan, July 14, 1951.

before their turn for antivenin injection. Conditions for carrying out the experiments as planned were even less practicable with *Bothrops* venom. In this case, 57 animals had died before antivenin treatment, though the venom dose was not certainly lethal, as proved by the survival of 3 of the 20 untreated control mice. Furthermore, it was impossible to perform the intravenous serum application in all of the animals scheduled for this test, as the circulatory collapse was already

TABLE 1

INTERVAL BETWEEN VENOM AND SERUM INJECTIONS	ROUTE OF ANTIVENIN ADMINISTRATION					
	Subcutaneous		Intravenous			
				Mortality		
	Dead before antivenin treatment	Mortality	Dead before antivenin treatment	When intravenous had to be replaced by subcutaneous route	On intravenous antivenin treatment	Of entire group
A. Venom of Bothrops jararaca *L-5 (0.05 mg/gm. = 0.01 ml/gm.) and antibothropic serum B-114*						
0	—	5/20	—	—	0/20	0/20
1	1	14/20	0	4/8	0/12	4/20
2	2	7/20	1	0/6	1/13	2/20
3	7	13/20	4	3/3	3/13	10/20
4	3	13/20	7	—	1/13	8/20
5	6	11/20	7	1/2	1/11	9/20
6	8	15/20	11	0/3	0/6	11/20
∞ (control)	—	17/20*	—	—	—	17/20*
B. Venom of Crotalus terrificus *L-4 (0.001 mg/gm = 0.01 ml/gm.) and anticrotalic serum C-100*						
0	—	0/20	—	—	0/20	0/20
1	0	4/20	0	—	0/20	0/20
2	0	11/20	0	—	3/20	3/20
3	0	13/20	0	—	2/20	2/20
4	0	16/20	0	—	4/20	4/20
5	0	18/20	2	—	8/18	10/20
6	2	20/20	2	—	12/18	14/20
∞ (control)	—	20/20*	—	—	—	20/20*

* Same controls used for both groups.

too advanced to encounter a vein. Excessive heating of the animals to provoke vasodilatation was, of course, contraindicated at this stage, since immediate death results from such a measure. These animals were injected subcutaneously but considered in the intravenous group. Also all mice which died before treatment were registered as dead in their proper lot, thus indicating that medical care had come too late in these cases.

The results of the experiments (Table 1) demonstrate the superiority of intra-

venous antivenin application of both of the venoms. It must be mentioned, however, that this method of treatment, even if employed immediately after venom inoculation, does not inhibit the development of local hemorrhage and necrosis by *Bothrops* venom. In the case of this venom, subcutaneous antivenin injection did not save all of the animals treated at once and was of questionable value if applied later. Intravenous treatment protected all animals when it was performed immediately, and most of those which were treated within 2 hours. After this period, the results obtained by this method were not very impressive. With the slowly acting venom of *Crotalus* all animals survived which received subcutaneous treatment at once or intravenous serum injections within one hour. Also mice which were treated subcutaneously within an hour or intravenously within 4 hours stood a good chance to survive. Further delay in antivenin treatment diminished the probability of recovery considerably.

In order to make a numerical comparison of the results possible, the data obtained in the experiments were statistically analyzed after the method of Bliss

TABLE 2

VENOM SPECIES	ROUTE OF ANTIVENIN INJECTION	INTERVAL IN HOURS BETWEEN VENOM AND ANTIVENIN INJECTIONS AT WHICH 50% SURVIVAL OCCURS		TEST FOR LINEARITY OF MORTALITY CURVE $\chi^2_{[4]}$
			Standard error	
B. jararaca	Subcutaneous	0:04	200:00	13.0
	Intravenous	3:35*	0:27	3.1
C. terrificus	Subcutaneous	1:56	0:13	2.4
	Intravenous	5:19	0:37	4.4

* 3:35 = 3 hours and 35 minutes.

(1938), the log of the interval between venom and antivenin injections being taken for x. Thus, the time after injection of the serum was determined which allowed 50 per cent of the test animals to survive. The figures obtained by this analysis (Table 2) confirm the superiority of the intravenous over the subcutaneous antivenin treatment. They demonstrate further that the increase of mortality by prolonging delay of serum application is a logarithmic function of time as evident from the satisfactorily low values of the χ^2 test, save in the subcutaneous experiments with *Bothrops* venom. Here, the large standard error of the result and the χ^2 test indicate the irregularity of the animal response and make it appear rather doubtful whether the subcutaneous antivenin treatment of the mice had any beneficial effect at all, except perhaps when given immediately after the venom injection.

COMMENT

Schöttler (1951) reported that the maximum venom yields observed in several thousand specimens of *B. jararaca* and *C. terrificus* on first extraction were 300 mg. The primary venom yield, however, is generally 40 to 70 per cent less than

subsequent ones, as demonstrated by Amaral (1928), Fairley and Splatt (1929), and Schöttler (1938). On the other hand, a venomous snake usually does not eject the entire contents of its venom glands in a single bite, but only from 25 to 75 per cent of the total, according to Amaral, or on the average 75 per cent, according to Fairley and Splatt, though the latter authors also occasionally observed complete discharge of the glands. As the two opposing factors compensate each other, they need not be considered.

If the proportions between the doses of venom and antivenin used in the present experiments are applied to snake bite in man, who might receive as much as 300 mg. of venom in a severe accident from either of the two species dealt with in this paper, 60 ml. of antibothropic and 3000 ml. of anticrotalic serum would be needed to correspond to this dose of venom. Even taking into account the fact that commercially available antivenins are about three times less potent than the special preparations used in this study, it may be concluded that very large but still practicable amounts of antibothropic serum will give a high degree of protection in severe cases caused by *Bothrops jararaca* if administered intravenously within 2 hours after the accident. Present or imminent circulatory collapse should be cared for by transfusions of large quantities of preserved human serum or plasma until blood pressure is restored to normal and maintained.

The results of the experiments with *Crotalus* venom demonstrate that serum treatment has a curative effect only in antivenin/venom proportions that are not applicable in case of severe snake bite by this species. This rather bleak outlook, however, is no reason to abandon the use of antivenin in accidents inflicted by that rattlesnake species. A certain venom-binding capacity of anticrotalic sera has been proved by animal experiments. Thus, quite a number of less severe cases, though fatal if untreated, may be cured by specific antivenin if the largest amounts injectable into man are administered by all routes as soon as possible after the accident.

SUMMARY

Mice were injected subcutaneously with venoms of *Bothrops jararaca* or *Crotalus terrificus* and treated with antivenins by intravenous or subcutaneous routes at varying intervals afterwards. The time interval between venom and antivenin injections at which 50 per cent of the animals survived, was 4 minutes if subcutaneous and 215 minutes if intravenous with the *Bothrops* venom, and 116 minutes if subcutaneous and 319 minutes if intravenous in the case of *Crotalus*.

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GEOGRAPHIC DISTRIBUTION OF *LATRODECTUS HASSELTII*

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Throughout a considerable portion of Southeast Asia, Australia and on many of the western Pacific islands *Latrodectus hasseltii* Thorell, 1870 is the counterpart, from a medical viewpoint, of the New World black widow spider, *Latrodectus mactans* (Fabr., 1775). As early as 1902 Cambridge had reported *hasseltii* from Australia, New Caledonia, New Guinea, the Loyalty Islands, the Bismark Archipelago, New Holland and the Philippines. Cambridge also cited records of the occurrence of this species in Africa, India, Burma, and the Persian Gulf area. Authenticity of African and Persian Gulf records may be questionable, since Smithers (1944) reported that at least one African specimen which had been identified as *hasseltii* was in reality a juvenile example of *Latrodectus indistinctus* Cambridge, 1905. As *Latrodectus tredecimguttatus* Rossi, 1790 is known to occur in North Africa and the Persian Gulf area, it may also be possible that immature specimens of this species have been erroneously identified as *hasseltii*. More recently Sloggett (1946) has reported occurrence of *hasseltii* on Morotai Island in the Halmaheras group, and at Koepang on Timor. The closely related *Latrodectus katipo* Powell, 1870 is known only on New Zealand, and may eventually be found to be synonymous with *hasseltii*. Accounts of the medical importance of *hasseltii*, including descriptions of symptoms produced by the bite, effects of venom on laboratory animals and methods of treatment of the bite have been summarized by Taylor and Murray (1946).

In this paper distribution of *L. hasseltii* in the Philippines is reviewed, several additional collection records are presented and the occurrence of this spider on Tinian Island in the Marianas group is reported for the first time. Taxonomic position of this species is discussed and a brief report is given on effects of *hasseltii* venom on laboratory animals. The writer has examined specimens of this spider from New Guinea, the Philippines and Tinian Island. The majority of these specimens were from the collections of the United States National Museum, and from the personal collection of the writer, although Dr. W. J. Gertsch very kindly loaned several specimens from the collections of the American Museum of Natural History

DESCRIPTION AND TAXONOMIC POSITION OF *L. hasseltii*

Latrodectus hasseltii, commonly known as the "redback" spider in Australia, is similar in size and general appearance to *Latrodectus mactans*. The body and legs are usually black in color, and the abdomen is marked dorsally with a longitudinal stripe. This stripe, which may be red, orange or yellow in color, is frequently constricted anteriorly, and in some specimens additional spots may

[1] Capt, MSC, USA.

lie in the mid-dorsal area anterior to the stripe proper. In juvenile specimens lateral markings extend from the central stripe, but are never separate from it, so that lateral spots are not seen in this species. In mature female specimens the ventral hour-glass marking typical of the genus may be absent, or represented only by one or two transverse bars. Although Cambridge (1902, 1902a) lists presence of acanthoid setae on the abdomen as a characteristic of *hasseltii*, he also figures and mentions specimens which lack such setae. Acanthoid setae have been absent from all specimens examined by the writer. On these specimens all setae have been relatively long and slightly curved. They may vary in size, but not in type, on any one specimen. Two specimens from Luzon possess dorsal abdominal setae which are relatively stouter than those on spiders from New Guinea and Tinian. No information is available concerning possible variation of setation with age of the specimen.

Cambridge (1902, 1902a) as well as Gerschman and Schiapelli in Sampayo (1942) considered *hasseltii* to be a subspecies of *tredecimguttatus*, differing from the latter only in color pattern. *Latrodectus katipo* Powell, 1870 has been considered distinct from *hasseltii* because the first pair of legs in this New Zealand species are relatively shorter than in *hasseltii* (Cambridge 1902, 1902a). Lack of acanthoid setae in *katipo* was also considered a distinguishing feature. Further study may reveal that neither of these characteristics is a valid criterion for separating the Australian and New Zealand forms into distinct species. Close relationship of *hasseltii*, *katipo*, *tredecimguttatus*, *menavodi*, *indistinctus* and *mactans* is revealed by the fact that females of these species have identical epigynes, and that palpal structure of males is alike (Cambridge 1902; Sampayo 1942; Smithers 1944).

DISTRIBUTION OF *L. hasseltii* IN THE PHILIPPINES

Published records of the occurrence of *L. hasseltii* in the Philippines have shown that this spider is rather widely distributed among the larger islands south of Luzon. It has been reported from Cebu by Plantilla and Mabalay (1935), from the Visayan Islands and Mindoro by de Jesus (1940), from Negros by Otanes (1932), and from Mindanao by de Asis (1934). The species has been reported only once from Luzon, where its occurrence in Tayabas (now Quezon province) on southern Luzon was recorded by Otanes.

Unpublished collecting data of Dr. F. del Rosario (pers. comm.), formerly with the United States Public Health Service in the Philippines, substantiated published reports and included a new record from Corregidor Island. Dr. del Rosario informed the writer that he had also found *hasseltii* on Cebu, where it had been described as a new species, *Latrodectus agoyangyang*, by Plantilla and Mabalay (1935). The specific name was chosen because of the resemblance of the abdomen of this spider to the black and red fruit of a plant called the "goyangyang." Plantilla and Mabalay did not designate a type specimen, but examination of both the written description and preserved specimens from Cebu has convinced the writer that they were dealing with *hasseltii* and not with a new species.

Philippine material in the collections of the United States National Museum includes specimens from Cebu, Negros, and Panay. The Panay specimens, both of which are females, are distinctive in that the legs are reddish brown rather than black. They were collected at Tibiao on May 11, 1918. No further collecting data were given. Another new distribution record for the species is afforded by two female specimens collected by the writer at Clark Air Force Base, Pampanga, Luzon. One specimen was found on 17 April 1950, the other on 3 May 1950. Both specimens were found under oil drums in a storage yard, where intensive search failed to reveal additional specimens. These two spiders were distinctive in possessing relatively stout dorsal abdominal setae, but lacked acanthoid setae. Neither specimen possessed a complete ventral hour-glass marking.

Effects of the venom of these two Luzon specimens on a white mouse and a guinea pig were similar to those reported by other workers (Keegan *et al.* 1950; Taylor *et al.* 1946). An adult white mouse bitten on the hind leg died in 25 minutes after exhibiting convulsive tremors, dyspnea, and paralysis of the hind quarters. Nearly identical symptoms were shown by a 435 gm. guinea pig, which was also bitten on the hind leg, and died in 45 minutes. Postmortem examination revealed hemorrhages in lungs, body cavity, and pericardium.

RECORDS OF *L. hasseltii* ON TINIAN ISLAND

Two lots of specimens from Tinian Island in the United States National Museum collection indicate a considerable extension of the range of *L. hasseltii*. One of these lots, collected by Downes and Cypert on August 25, 1945, contains 25 specimens: 3 males, and 22 females, including several juvenile specimens. The remaining lot consists of 64 female specimens, all but 14 of which are immature. These were collected by C. B. Worth in 1944. It is not known whether *hasseltii* was present on Tinian before the war. This discovery of the occurrence of the species on Tinian makes its range comparable with that of *mactans*, which is found not only on mainlands of North and South America, but also occurs on the eastern Pacific islands, and as far west as the Hawaiian Islands, where it is now well established. All specimens from Tinian possessed complete hour-glass markings ventrally. They also differed from the Luzon specimens in that the dorsal abdominal setae were relatively long and slender, and very much like those of *L. mactans*, or in some cases, of *L. geometricus*.

SUMMARY

1. Distribution of *L. hasseltii* is reviewed and new collection records from Corregidor Island, Panay, Luzon, and Tinian Island are reported.

2. Taxonomic position of *Latrodectus hasseltii* is discussed and a brief description of the species is given.

3. Effects of the venom of two Luzon specimens on a white mouse and a guinea pig were similar to those reported by other workers. Symptoms produced by the bite of an adult female *hasseltii* included convulsive tremors, dyspnea and paralysis. The white mouse died 25 minutes after receiving a bite on the hind leg and the guinea pig died 45 minutes after being bitten on the same area.

Postmortem examination revealed hemorrhages in lungs, body cavity and pericardium.

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CORRESPONDENCE

New York, N. Y.
September 10, 1952

To the Editor:

I should like to bring to the attention of the readers of the Journal an opening we have with the Liberian Institute of the American Foundation for Tropical Medicine as Resident Director. As you doubtless know, the Liberian Institute is now in its initial phase of operation and has developed to the stage where we wish to place a full-time Director in charge.

The Liberian Institute is located in Harbel, Liberia, West Africa and is established as a research and training center in tropical medicine. The plant consists of a large, two story 2-wing laboratory building with twenty-four individual lab rooms. These lab rooms are furnished with modern laboratory furniture and each is equipped with water, gas, electricity and vacuum. In conjunction with the lab, there are several appurtenant buildings for animals, garage, workshop, etc.

Staff residential quarters consist of three 2-bedroom houses completely furnished and attractively constructed. Others will be built as funds are available and the need arises. For the native staff help, a twelve section dormitory has been provided and another is planned.

Liberia has an ideal tropical climate and those who live there enjoy it. The Institute is located but two miles from Roberts Field, international airport for West Africa and some five miles from the Firestone Plantations Company where recreational facilities and a hospital are available. The basis of cooperation between Firestone Plantations and the Liberian Institute is excellent.

The Resident Director we seek should be in the age range of 30 to 50. He should be a graduate of a recognized Medical School, School of Public Health or their equivalent and have pursued studies in tropical medicine, pathology, bacteriology, parasitology, entomology, biological chemistry and pharmacology. He should have done some research in this field and demonstrated a capacity for administration.

The salary range is $10,000 to $12,000 depending upon experience. We seek a single man by reason of present accommodations but if a qualified applicant is married and has small children, he could become adjusted to living there but with more difficulty. We shall provide a house at no cost, ocean transportation, a car in Liberia and other conveniences. Income taxes are refunded after a person is out of the country for 18 months. The position is a permanent one with the first tour of duty for a two year period. After that, the contract will be renewed on a year to year basis. Paid vacations with expenses back to the U. S. will be given on the basis of one month for each year of service.

For those interested in applying, I suggest they send background information to the Executive Director of the Foundation:

Mr. Ernest F. Gamache
American Foundation for Tropical Medicine
345 Madison Avenue
New York 17, New York

With appreciation for your cooperation,

Very truly yours,
THOMAS T. MACKIE, M.D.
Chairman, The American Foundation for Tropical Medicine

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

Zinsser's Textbook of Bacteriology. By David T. Smith, Norman F. Conant, Joseph W. Beard, Hilda Pope, D. Gordon Sharp and Mary A. Poston, of Duke University School of Medicine. 10th Edition. 1012 pp. 330 illustrations. New York, Appleton-Century-Crofts, Inc. 1952.

Comparability of Statistics of Causes of Death According to the Fifth and Sixth Revisions of the International List. Bulletin of the World Health Organization, Supplement 4. Geneva, W. H. O., Palais des Nations, 1952. Price 2/9; $0.50; Fr. fr. 160; Sw. fr. 2.–

Biologie d'Anopheles gambiae. Recherches en Afrique-Occidentale Française. Serie de Monographie No. 9. By M. H. Holstein, Chargé de recherches de l'Office de la Recherche Scientifique Outre-Mer, Paris. (English Edition in preparation) 176 pp. Geneva, W. H. O., Palais des Nations, 1952. Price 10/–; $2.00; Fr. fr. 640; Sw. fr. 8.–

Microbial Growth and its Inhibition. First International Symposium on Chemical Microbiology. W. H. O. Monograph Series No. 10. Mixed language Edition containing articles in English or French with summaries in both languages. 286 pp., 67 figures. Geneva, W. H. O., Palais des Nations, 1952. Price 15/–; $3.00; Fr. fr. 960; Sw. fr. 12.–

Expert Committee on Mental Health, Alcoholism Subcommittee, Second Report. W. H. O. Technical Report Series No. 48. (Available also in French). 39 pp. Geneva, W. H. O., Palais des Nations, 1952. Price 2/–; $0.25; Fr. fr. 80; Sw. fr. 1.–

Photosensitization in Diseases of Domestic Animals. Review Series No. 3 of the Commonwealth Bureau of Animal Health. By N. T. Clare, Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand. 58 pp. with 8 illustrations. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England, 1952. Price 7/6.

Précis de Mycologie, Mycologie Générale, Humaine et Animale, Techniques. By M. Langeron and R. Vanbreusegheur. Preface by Prof. Edmond Sergent. 2nd Edition, revised and amplified. 701 pp. with 461 illustrations. Cloth. (Collection de Précis Médicaux) Masson et Cie., 120 boulevard Saint-Germain, Paris 6e, France. Price: Paper, Fr. fr. 4800; Cloth Fr. fr. 5400.

International Sanitary Regulations. Proceedings of the Special Committee and of the Fourth World Health Assembly on W. H. O. Regulations No. 2. Official Records of the World Health Organization No. 37. (Also published in French). 443 pp. Geneva, W. H. O., Palais des Nations, 1952. Price $2.25; 16/3; Fr. fr. 720; Sw. fr. 9.

Third Report of the Expert Committee on Insecticides. W. H. O., Technical Report Series No. 46. Geneva, W. H. O., Palais des Nations, 1952. Price 2/–; $0.25; Sw. fr. 1.

BOOK REVIEWS

International Health Organizations and their Work, by NEVILLE M. GOODMAN, M.D., F.R.C.P., D.P.H., formerly on the Health Committee of the League of Nations; British Delegate to the International Office, Paris; Director of Health for UNRRA in Europe; now Assistant Director General (Acting), WHO and Lecturer on International Health, London School of Hygiene and Tropical Medicine. pp. 327, with 53 illustrations, cloth. Philadelphia-New York, The Blakiston Co., 1952; $6.50.

The author's long and notable career as a prominent leader in international health qualifies him, as few others are qualified, to prepare this work. Throughout its thoroughly absorbing 315 pages there are numerous reflections of his intimate knowledge of the events and, oftentimes, personalities concerned and it is these that have enabled him to make the book far more than a careful chronology—although it is this also.

The coverage of each important phase of development from the problems of quarantine in the early 18th century to the 3rd World Health Assembly in 1950, the generous use of appendices that provide relevant details from important basic documents and records and the extensive system of references make this book highly valuable to any student of international health whether he be engaged in academic pursuit of some aspect of this field or actively engaged in it as a health worker or statesman. It would serve admirably as a text book for the teaching of this subject at the university level. Indeed, it is so well written and the subject matter so clearly presented that it should prove of equal value to nonprofessional people who are interested in this important aspect of international cooperation.

The treatment of the developments of the past three decades is especially complete and draws from the author's own experiences in such activities as those of the International Office of Public Health, the Health Committee of the League of Nations, and more recently in the World Health Organization. Not only does he trace, and make comprehensible, the evolution and role of these and other major organizations but he also accomplishes the difficult task of clarifying the relationships among them and between them and other international agencies that are in the health field or in fields related to it.

EDWARD S. ROGERS

Textbook of Clinical Parasitology, Second edition, by DAVID L. BELDING M.D., Professor of Bacteriology and Experimental Pathology, Emeritus, Boston University School of Medicine, 1139 pages with numerous text illustrations and summary tabulations. Appleton—Century—Crofts, Inc., New York, 1952. $12.00.

The second edition of this book should be as generally well received as was the first edition. It is a valuable text for medical students and reference book for physicians and others interested in parasites of man. However, despite the misleading title "Clinical Parasitology" it is not a clinical text. In common with other books with the same or similar titles, it features the morphology and biology of the protozoa, helminths, and arthropods which infect and infest man. That coverage is, however, rather better presented for the student than is the case with a number of other books in the field. The numerous illustrations set this book apart from most other texts. They have been selected to illustrate salient points and are well executed; the labeling of these illustrations is excellent and alone puts the book in a unique position in the field. Oversimplification and minor errors in such illustrations do not detract from their significant value.

The numerous tabulations in the introductory chapters (pp. 1–62) are useful but inevitably are misleading. For example in table 3 (pp. 10–11), titled "Principal Hosts...", to list man as the first of a series of definitive hosts for *Balantidium coli*, *Trypanosoma cruzi*, *Trichinella spiralis*, *Dipylidium caninum*, *Hymenolepis nana*, *Fasciola hepatica*, and *Opisthorchis felineus* and as the first of the intermediate hosts of *Echinococcus granulosus* can stem only from anthropological egotism or ignorance of the true occurrence of these parasites and misleads the uninformed reader. To include man as a definitive host of *Ancylostoma brazilense* is debatable to put it conservatively. Similarly other such summary tables are misleading if taken without reference to the detailed discussions. Nevertheless the tables are useful and little harm is done to the reader whose interest is more than casual.

A book bearing in the title the term 'Clinical' would be expected to contain a critical comparison of clinical manifestations and adjunct clinical laboratory procedures as an aid to differential diagnosis. These are notably lacking for the obvious reason that they are difficult if not impossible tasks with infections which are often subclinical. Perhaps the solution would be to change the misleading name in subsequent editions.

Epidemiology is as conspicuously inadequate in this book as in other texts in medical parasitology but perhaps is not required.

Despite the criticisms the book is well written, instructive, and well set up by author and publishers. It is the reviewer's choice for classroom use.

GILBERT F. OTTO

Introduction to Malaria Problem in India, by Lt. Col. B. L. Raina, Army Medical Corps; with an introduction by Dr. Jivraj N. Mehta. 134 pp., illustrated. Bombay, New Book Co., Ltd., 1951. Price Rupees 7/12/–.

This small book, an elaboration of a prize essay, while making no pretense to cover completely the malaria problem of India nevertheless gives a panorama of it that cannot elsewhere be found in a single brief volume. This is its chief value. But it is to be hoped that the book's reiteration of the thesis, propounded years ago by the Malaria Institute of India that India's malaria problem can, and ought to be, controlled, may serve to stimulate an all-out attack on malaria by Indian governments.

Malariologists, editors and scientists in general, can find much in text to which exception may be taken: The use of "micro" and "macro" as separate words associated with gametocytes (p. 12); and the use of "specie" as the singular of "species" (p. 38) are examples. On page 13 it is implied that quotidian fever is always due to infection with more than one species of parasite. On page 73 it is suggested that antilarval work may be cheaper than residual spraying in the absence of epidemic conditions. On pages 97–98 plasmoquine is recommended as a gametocide for malaria prevention, although on page 79 it is stated that plasmoquine should be given only to hospitalized patients. On page 92 it is stated that ". . . every rupee spent (on anti-malaria work) will mean thousands of rupees saved . . .", an estimate which seems unduly optimistic.

The appendix, which might have been incorporated in the text, since it gives repetitious as well as useful additional information, comprises one-fourth (31 pages) of the book.

These criticisms may happily be linked to the adage: "Good things come in small packages." The book makes no pretensions of being a textbook and as an introduction to the subject treated it serves its purpose well.

R. B. Watson

A Color Atlas of Morphologic Hematology with a Guide to Clinical Interpretation, by Geneva Daland, Edited by Thomas Hale Ham; Illustrated by Etta Piotti. From the Thorndike Memorial Laboratory, Boston City Hospital. 74 pp. 23 illustrations, 14 in color. Cambridge, Harvard University Press, 1951. Price $5.00.

This color atlas is a useful reference for hematologic technicians and medical students. The backbone of this little volume is formed by fourteen lithographed color plates illustrating the normal morphology of the blood cells and their characteristic appearance in various diseases. The maturation of the different series of cells is clearly diagrammed. Technique for cell counting, for blood smear interpretation and for obtaining supportive data are described. The nomenclature of the different cells used in this text does not conform to that recommended by the Society of Clinical Pathologists, but is accurate by definition and intrinsically consistent.

It should be noted however that this atlas monograph does not emphasize cellular pattern and does not show the appearance of the bone marrow in the normal or disease states. Since most hematologic problems involve marrow studies, such information is needed by students and practicing physicians. The scope of the book has thus been limited by the authors. However, the material covered, the format of the book and the color plates are as a whole good.

Jonah G. Li

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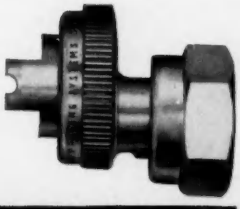

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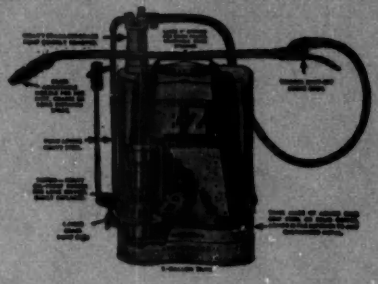

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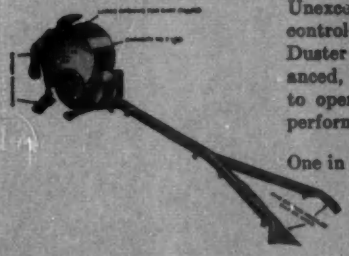

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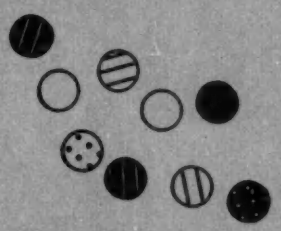

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